

PEANUT ALLERGY:
A PROSPECTIVE STUDY OF THRESHOLDS,
CO-FACTORS, MEDIATORS AND SEVERITY

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Thesis submitted for the degree of *Doctor of Philosophy*

October 2017

SUMMARY

Peanut allergy: a prospective study of thresholds, co-factors, mediators and severity

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Peanut allergy is a public health concern which affects a significant proportion of the population. Accidental exposure to peanut can cause severe and fatal reactions in peanut allergic individuals and currently their only safeguard is to practise careful avoidance. Identification and protection of at-risk members of the allergic population is critical in managing this life-threatening condition. This thesis produces key data to enable this.

A prospective study was performed on 60 peanut allergic participants to determine thresholds of reactivity to peanut using oral challenges with incrementally increasing amounts of peanut protein. Following a double-blind placebo-controlled peanut challenge, participants received three further peanut challenges, two with co-factors: sleep deprivation and exercise, and one without. Severity was measured using a numerical scale derived from symptoms and serum tryptase was measured at each challenge. A total of 187 challenges were performed.

Findings were that the median amount of peanut protein which induces a reaction in 10% of the population (ED10) was 12.3mg (95% CI 7.3,20.4) equivalently this suggests that 90% of the allergic population will not react to doses below this level. Both sleep deprivation and exercise have a significant effect on lowering reaction threshold (ED10), by 5 times and 2.5 times respectively. Separately there is a reduction in threshold with successive challenges.

Co-factors also significantly increased symptom severity during challenge reactions. In particular sleep deprivation significantly increased the severity of gastrointestinal symptoms suggesting that a stressful stimulus may affect intestinal permeability. Evidence was provided for the importance of asthma as a risk factor which increased the severity of respiratory symptoms during reaction. Using a novel visual analogue scale for measuring the participant's perception of severity, a poor correlation was observed between the participant's perception of the reaction and the overall numerical severity score, suggesting that participants misperceive severe symptoms. This thesis provides the first data showing that symptom patterns in repeated challenges show a high degree of homogeneity within individuals, but importantly that this symptom homogeneity is also observed across individuals.

Lastly the utility of serum tryptase in identifying food allergic reactions has been disputed previously. This thesis provides evidence of its value and identifies a rise cut-off of 30% as being diagnostic of a food allergic reaction, but cautions that acute levels must be compared with baseline as this rise may occur within the normal range.

DECLARATION

I declare that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. This work has not been submitted for any other qualification

STATEMENT OF LENGTH

This dissertation does not exceed the limit of 60000 words as set by the Degree Committee of the School of Clinical Medicine and Veterinary Medicine

ACKNOWLEDGEMENTS

I would like to thank Dr Pamela Ewan and Dr Andrew Clark for giving me the fantastic opportunity to work on this study and for their invaluable advice, unfailing support, formidable teaching and patience over the course of this study. I am indebted to Yvonne King for her extremely kind and diligent assistance in coordinating and arranging appointments for study participants and for her help with challenges and data entry. I am grateful to Professor James Wason and Dr James Dowey for their help with statistics in this thesis. I am privileged to have worked with an excellent team on the Cambridge Clinical Research Facility and thank them for their excellent and safe care of study participants. I am grateful to Dr Sabita Islam and Dr Loraine Foley for their help in preparing challenge meals, sample handling and data cross-checking. I wish to acknowledge the Food Standards Agency, UK for funding this study.

Thank you, in particular, to Drs. Lucy Foster, Pav Ghataorhe, Dani Lux, Rishma Maini, Sarah McRobbie, Brinnie Selvarajah, Priya Sellaturay, Rebecca Tibbott, Nikki Weir and Mrs Camilla Serino for their advice, encouragement and loyal friendship.

I am grateful to my mother, father and sister for inspiring me from a young age to be like them and for their endless love and support throughout.

Finally, this thesis is dedicated to my husband and best friend James, as without him this would have been an impossible feat.

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GLOSSARY

APC	antigen presenting cell
CRF	Clinical Research Facility
DBPCFC	double blind placebo controlled food challenge
ED	Elicitation dose
FDEIA	Food dependent exercise induced anaphylaxis
FEV	Forced Expiratory Volume
IgE	Immunoglobulin E
IgG4	Immunoglobulin G4
IM	intramuscular
IQR	Interquartile range
ICSA	Interval Censored Survival Analysis
IV	intravenous
LOAEL	lowest observed adverse effect level
MED	minimum eliciting dose
µg	microgram
mg	milligram
NOAEL	no observed adverse effect level
NRES	National Research Ethics Service
NSAID	non-steroidal anti-inflammatory drug
OFC	oral food challenge
PAL	Precautionary Advisory Labelling
QOL	quality of life
ROC	Receiver Operating Characteristics
SPT	skin prick test
Th2	T helper 2
TMG	Trial Management Group
TRACE	Threshold Reactivity and Clinical Evaluation Study
TSC	Trial Steering Committee
VAS	Visual Analogue Scale
VO2	Maximum rate of oxygen consumption
WDEIA	Wheat dependent exercise induced anaphylaxis

Thesis Outline

Peanut allergy is a significant public health concern as peanut has the propensity to cause severe and fatal reactions following inadvertent consumption by peanut allergic individuals. Currently there is no licensed, disease-modifying therapy for peanut allergy and the mainstay of treatment is to advise careful avoidance, and provide emergency medication.

Unfortunately, this approach is not watertight and accidental exposures still occur. Quality of life is adversely affected, and is related to fear of reactions, and limitations in food choices, made worse by ambiguous precautionary food labelling.

To engage with this issue and whilst we wait for new therapies to emerge, a few strategies may be considered. Firstly precautionary food labels, which are currently not fit for purpose, can be improved to assist food allergic consumers to broaden food choice and practise safe allergen avoidance. Secondly attempts can be made to more accurately stratify patients at high risk of a severe reaction, so that they can be better advised to avoid high-risk situations. Lastly, laboratory tests to help emergency clinicians diagnose patients undergoing acute allergic reactions can be improved ensuring that allergic patients are correctly identified and referred for specialist care.

A prospective study of experimentally induced systemic allergic reactions in peanut allergic participants forms the basis of this thesis. As one of the lead investigators on the study, I personally performed 187 peanut challenges and witnessed first-hand the ensuing allergic reactions, and treated them. Using the experience of performing these challenges, I have explored the three areas described above. The first theme is a study on thresholds in peanut allergy and seeks to establish a population reactivity threshold for allergic individuals, with the ultimate aim of informing reference doses for allergens. These can be used by food regulators and the industry to improve precautionary labelling accuracy. The second theme examines in great detail the severity of peanut allergic reactions and symptom patterns within and across participants to elucidate whether there are common and predictable features of severe reactions. Incorporated into these sub-studies, in an attempt to replicate real life exposures, is an examination of the effect of two co-factors: exercise and sleep deprivation on the threshold and severity of allergic reactions. The final theme focuses on the utility of measurement of serum tryptase, a mast cell mediator, in the diagnosis of acute peanut allergic reactions.

CHAPTER 1

Literature Review

Part 1: General Overview

An allergy is a hypersensitivity reaction which the immune system launches against a substance. Allergy can lead to a variety of diseases which manifest in different organ systems. These conditions include life threatening anaphylaxis, food allergy, urticaria, certain forms of asthma, eczema, rhinitis, drug and venom allergy. The global burden of allergic disease is considerable and it is estimated that at least 20% of the population will suffer from an allergic disease at some point in their lives.¹ There are suggestions that the prevalence of allergic disease is increasing. Two studies which used similar methodologies and the same population performed in the late 1990s and in the early 2000s demonstrated a 2.5 fold increase in the incidence of anaphylaxis (21 cases per 100,000 person-years and 49.8 cases per 100,000 person-years respectively).^{2,3} Studies in the United Kingdom which have examined databases of hospital discharges over time have shown a sevenfold increase in patients who had been hospitalised due to allergic disease.⁴ This upsurge in patients suffering from allergic disease is being referred to as the ‘Allergy Epidemic’.

Reasons behind the ‘Allergy Epidemic’ have yet to be properly elucidated. In the late eighties the ‘Hygiene Hypothesis’ was proposed by the British physician, Strachan. Quite simply this stated that early life infections are needed for normal immune system development and a reduced number of these infections provided a predisposition to allergic diseases.⁵ However in recent times this has been redefined as the ‘Biodiversity Hypothesis’ which claims that the excessive Western hygienic lifestyle reduces microbial exposure and alters the early colonisation of the infant gut, disturbing the normal tolerance development of the immune system.⁶ This theory has been supported by several epidemiological studies. One study showed lower diversity of gut microbiota in the first week of life is associated with atopic eczema at 18 months.⁷ A further study showed that the probability of developing asthma in farming children is inversely related to the range of exposure to environmental bacteria and fungi. It has also been postulated that an infant’s diet early in life can potentially influence atopic sensitisation later on. In a Finnish birth cohort it was observed that less food diversity in the first year of life might increase the risk of allergies and asthma in childhood.⁸ Thus a reduction in ‘antigenic burden’ can potentially reduce the stimulation of the immune system in genetically predisposed individuals which in turn may lead to a dysregulated

immune response and allergy. Food allergies in particular are noticeably increasing in prevalence in westernised populations.

Allergy mechanism

The term allergy first appeared in the medical literature in 1906 when the Paediatrician Clemens von Pirquet noted that the response to some antigens resulted in damage to the host rather than a protective response.⁹

The allergic response occurs in 3 phases: sensitisation phase, effector phase and the late phase response. In the sensitisation phase the allergen is encountered for the first time and presented by an antigen presenting cell (APC) to a naïve CD4⁺ T helper lymphocyte. These cells are instrumental in orchestrating the immune system. These effector Th2 cells produce cytokines IL3, IL4 and IL13 and stimulate B cells to induce class switching of immunoglobulin heavy chain to the ϵ type. This stimulates the production of allergen-specific IgE antibodies. Secreted IgE then circulates in the blood and binds to high affinity IgE receptors (Fc ϵ RI) on the surface of mast cells and basophils. In the effector phase, a new encounter with the allergen causes cross-linking of the IgE bound to Fc ϵ RI surface receptor. IgE cross linking induces aggregation and migration of receptors into membrane lipid rafts, followed by phosphorylation of ITAM motifs by associated tyrosine kinases. Adapter molecules then latch onto phosphorylated tyrosine residues and initiate signalling cascades culminating in enzyme and/or transcription factor activation. Fc ϵ RI signalling leads to mast cell and basophil (1) degranulation of vesicles containing inflammatory mediators, (2) expression of inflammatory cytokines and 3) conversion of arachidonic acid into leukotrienes and prostaglandins. The mediators released by mast cells are preformed and granule associated. They include histamine, proteases and heparin. These act on different cell types producing rapid inflammatory responses including vasodilatation, increase in vascular permeability and smooth muscle contraction. These produce the symptoms typical of an allergic reaction including urticaria, angioedema, bronchospasm, vomiting, diarrhoea and hypotension. Hours after the immediate phase of an IgE-mediated response, mediators released during the course of the reaction induce localised inflammation called the late phase reaction. Cytokines released from mast cells particularly TNF α and IL1 increase the expression of cell adhesion molecules on venular endothelial cells thus facilitating the influx of neutrophils, eosinophils and Th2 cells. Eosinophils play a critical role in this late phase response. Eosinophil chemotactic factor released by mast cells in the initial reaction attract

large numbers of eosinophils to the affected site. Cytokines released at the site including IL3, IL5 and GM-CSF contribute to the growth, differentiation, activation and increased survival of these cells. On being activated, eosinophils release inflammatory mediators such as eosinophilic cationic protein (ECP), major basic protein (MBP), leukotrienes and prostaglandins that contribute to the extensive tissue damage typical of the late phase reaction. Clinically this manifests as prolonged mucus secretion, oedema formation and persistent bronchial hyper reactivity. Neutrophils similarly involved in the late phase reaction are attracted by neutrophil chemotactic factor released from degranulating mast cells. Granule contents of neutrophils include lytic enzymes, platelet activating factor and leukotrienes.

Food allergy

Food allergy encompasses a spectrum of conditions characterised by abnormal immunological responses to food. IgE mediated reactions to food are the most common and include a number of symptoms which are rapid in onset including oropharyngeal pruritus, flushing, urticarial or angioedema, nausea, vomiting, abdominal pain, bronchospasm, cardiovascular collapse and rarely death. Diagnosing IgE mediated food allergy relies on a careful history to elicit the allergen in question, skin prick testing, serological confirmation of ¹⁰the presence of IgE and where appropriate oral food challenge to the allergen.

Food allergy is a significant public health concern for numerous reasons including increasing hospital utilisation, increasing associated medical costs¹ and an increasing burden of care on immediate families. Moreover, a food allergy can place a significant burden on an individual by restricting their daily food choices and travel plans.¹¹ Indeed several studies have shown that having a food allergy impairs an individual's quality of life.¹²

How widespread is the problem? Globally around 200-250 million people suffer from food allergies.¹³ Unfortunately, estimating the prevalence of food allergy is a challenge because most of the data on prevalence is based on simple questionnaires, some unvalidated, which use self-reporting and not objective tests to document true allergic reactions. The gold standard of diagnosing allergy is through a double blind placebo controlled food challenge. In this test, a third party prepares two foods for testing: one placebo food and one active challenge food which should be indistinguishable. These are then given to the patient separated by an interval. This procedure, however, is expensive and time-consuming and has the potential to trigger adverse reactions. Furthermore, variations in food allergy definitions and markers (skin prick test positivity or serum IgE positivity) and study methodology leads

to inconsistent prevalence estimations. Food allergy prevalence based on self-reporting is approximately 12-13% compared with studies using oral food challenge, 3%.^{14,15,16} Most studies, however, are in agreement that the prevalence of food allergy has increased in recent years.^{17,18} Recent hospitalisation data of food induced anaphylaxis has suggested that the incidence rate has been rising especially in children under 4 years of age. A recent review of the literature indicated a prevalence of 2-10% of the population¹⁹ with the prevalence being more common in children compared to adults (8% versus 5% in the USA).^{14,20} The development of IgE and sensitisation to food proteins is usually the first manifestation of allergic disease i.e. the commencement of the Allergic March. Risk factors for the development of food allergy include the presence of eczema and a positive atopic family history. Other potential risk factors which have been suggested include ethnicity (increased in Asian and black compared to white children), geographical factors, genetics (familial associations with HLA and specific genes), Vitamin D insufficiency, the timing and route of allergen exposure, obesity (being an inflammatory state) and increased use of antacids (reducing breakdown of allergen in the gut).¹⁴

Peanut allergy

Peanut (*Arachis hypogea*) allergen attracts a lot of attention as it is widely consumed and has the propensity to cause severe and even fatal reactions.

Peanut allergy is no exception with regard to the general rising trend in food allergy prevalence. Studies from the United States, U.K, Canada and Australia place prevalence rates of peanut allergy at 1-2% for children and 0.6% for adults. The prevalence does seem to vary in different regions worldwide, for example in France (0.3-0.7%), Denmark (0.2-0.6%) and Israel (0.04-0.17%) and in Asian countries where peanut allergy is exceedingly rare.^{21,22,23,24} Reasons for this variation are not fully understood but possible explanations include differences in the timing of introduction of peanut into the diet, peanut preparation practices (roasted versus boiled), differences in exposure to sunlight with regard vitamin D levels or variation in the prevalence rates of atopy in general. Anaphylaxis fatality registers are indicative of the severity of peanut allergy. In the United States peanut was implicated as a trigger in 59% of 63 deaths,^{25,26} however in a U.K report, peanut was less frequently implicated with peanut being a cause in 19% of 48 fatalities.²⁶ Currently the standard of care for management of peanut allergy is allergen avoidance and a well primed emergency treatment plan. This provides great impetus for research in this field to develop better

strategies for treatment and prevention.

Risk factors for the development of peanut allergy

The fact that a family history of peanut allergy for example, in a parent or sibling, renders a child with a 7 fold increase in the risk of peanut allergy suggests that there may be a genetic component to the development this disease.²⁷

Race and migration may also play a role. Panjari et al showed that children who were born in Australia to Asian-born mothers were more likely to have a nut allergy than non-Asian children. However children who were born in Asia and subsequently migrated to Australia were at a decreased risk of nut allergy.²⁸ These findings raise the possibility of gene-environment interactions.

It has been established that sensitisation to peanut allergen can occur transcutaneously and therefore it follows that eczema is a risk factor for peanut allergy. In a mouse model, mice which were subjected to tape stripping, a surrogate for excoriations in atopic dermatitis, developed inflammation and subsequent sensitisation to topically applied allergens.²⁹ The Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study found that children who developed peanut allergy by the age of 5 were more likely to have had severe eczema in the first 6 months of life and to have been treated with Arachis oil for dry skin.³⁰ Furthermore mutations in filaggrin, a protein important in regulating transepidermal water loss across the skin barrier, have been shown to be associated with peanut allergy and sensitisation.³¹ Also mice with filaggrin mutations are more susceptible to becoming sensitised to an allergen when it is applied topically.³²

Environmental allergen exposure may also play a role. In a case-control study by Fox, peanut consumption in the home was used as a marker for environmental exposure. They showed a clear dose response relationship between household peanut consumption (using a validated questionnaire) and the risk of peanut allergy development in young children.³³ In a further study Brough et al actually measured the presence of peanut allergen in household dust and found that high levels of peanut in household dust were associated with an increased risk of sensitisation and likely peanut allergy in children with atopic dermatitis particularly in children with severe atopic dermatitis.³⁴

Since peanut allergy presents in infancy, the influence of maternal and infant diet has been examined with regards to the development of allergy. Sicherer et al examined an atopic cohort of 503 infants with no previous diagnosis of peanut allergy and demonstrated that maternal ingestion of peanut during pregnancy was associated with high levels of peanut sensitisation, however when this was later adjusted for household exposure, the effect on peanut allergy was no longer significant.^{35,36} There is debate around the role of breast feeding and studies have failed to show a consistent protective effect.^{37,38}

The timing of introduction of food allergens and the development of food allergy could also potentially be important and there are studies to support this. A cross sectional study by Du Toit found that the prevalence of peanut allergy was 10 fold higher in UK Jewish children (1.85%) compared to Israeli Jewish children (0.17% $p < 0.001$) Possible explanations for this include differences in the median monthly consumption of peanut in Israeli infants aged 8-14 months (7.1g) compared to 0g in the UK. Furthermore, peanut is introduced earlier in Israel. Therefore early introduction of food allergens with more frequent consumption in the early stages of infancy may have a protective role and be instrumental in inducing oral tolerance to food allergens.³⁶ These theories have recently been tested in two landmark studies. Du Toit et al performed a randomised controlled interventional study to test the timing of peanut introduction on the rate of peanut allergy development. The authors found that 17.2% of subjects in the avoidance group developed peanut allergy compared to 3.2% of the early introduction group.³⁹

Therefore although environmental exposure to peanut does seem to be associated with an increased risk of peanut sensitisation and peanut allergy in high risk groups exposure via the oral route has a more important role in the individual developing peanut tolerance, possibly as a result of increased allergen dose via the oral route and an increased regularity of exposure versus the environmental and transcutaneous route.

The acquisition of natural tolerance is complex and requires further understanding. However immunological changes during immunotherapy have been well defined. These involve the development of peripheral tolerance by the promotion of regulatory Treg and Tr1 cells. These directly or indirectly suppress pro-inflammatory cells such as mast cells, eosinophils or basophils by formation of specific IgG4 followed by reduction in specific IgE.

Natural history of peanut allergy

Peanut allergy usually presents early in life. Several studies performed in the US over the last 15 years have shown that it frequently presents by the second year of life. Usually the first exposure to peanut occurs between the ages of 12 and 22 months and the onset of the first reaction is usually at 14-24 months. The first reaction usually occurs on the first known exposure in 75-80% patients.^{40,41,42 43}

Twenty percent of children with peanut allergy and 10% with tree nut allergy outgrow these allergies.^{44,45} Recent studies examining the natural history of peanut allergy continue to support this. Arshad et al observed natural resolution of peanut allergy in 17% subjects ages 4 to 10 and 26% ages 10 to 18 in a birth cohort observed through to age 18.⁴⁶ A longitudinal study of children diagnosed with peanut allergy at 1 year of age showed that peanut allergy resolved by age 4 in 22% of children and also found that decreasing SPT wheal size predicted tolerance to peanut and an increasing SPT wheal was associated with persistence of allergy.⁴⁷ Low rates of natural resolution means that the majority of peanut allergic patients remain peanut allergic through adulthood.

There are few studies of peanut allergic adults. Savage et al reported that adults with late onset disease (age >10 years) tended to have milder symptoms, smaller skin prick tests and lower levels of serum specific IgE to peanut than adults with early childhood onset. It has been proposed that that late onset adult disease is associated with sensitisation to cross reactive pollen allergens rather than primary allergens.⁴⁸

Peanut allergens

An allergenic protein is a molecule that has the ability to induce sensitisation and to trigger a reaction. There are multiple peanut varieties however the allergenic proteins are conserved among them and are found in the cotyledon (seed leaf). Peanut allergy can either be caused by primary sensitisation to the disease eliciting food allergen or it can occur as a result of primary sensitisation to inhalant allergens and subsequent IgE cross-reaction to homologous proteins in food (minor allergens). Allergens are considered major if they are recognised by the serum IgE of greater than 50% of the allergic population.

Seed storage proteins, which form the major allergens within peanut, are present as one or more groups of proteins in large amounts in seeds to provide a store of amino acids for use during germination and seed growth. They are extremely stable against denaturation from

heat, acidity and proteolytic activity. As a result, these allergens are presented to immune cells in the gut in an almost intact form and therefore usually induce severe reactions. Several allergens have been identified in peanut including the cupin superfamily members which form the major seed storage proteins of peanut known as the 7s and 11s seed storage globulins (Arah1 and Arah3 respectively). The function of these proteins within the peanut plant is to inhibit fungal growth and deter insect predators. Arah1 belongs to the vicilin (7s) family of seed storage proteins. It is a glycoprotein and contains 23 independent Ig-E binding epitopes.⁴⁹ Burks et al performed 3-dimensional modelling and demonstrated that Ara h 1 forms stable homotrimers with allergenic sites clustered into two main regions.⁵⁰ The internal location of the IgE binding regions explains the relative weak activity of native Ara h 1 in cross linking IgE and the strong binding of IgE to denatured monomers.^{51,52} Ara h 2, 6 and 7 belong to the prolamin superfamily and include the 2S albumin fraction. These are considered to be plant defence-related proteins. The biological plant protective functions of many of these proteins contribute to their resistance to degradation as well as to their overall allergenicity.⁵³ Arah2 contains 10 independent IgE-binding epitopes stretching throughout a linear structure.⁵⁴ There are two isoforms Ara h 2:01 and Ara h 2:02. The larger isoform, Arah 2:02 contains 12 extra amino acids including duplication of a strong IgE sequence and hence binds more IgE.⁵⁵ Ara h 6 is 59% homologous to Arah2 but is 2-4 kD smaller. It is heat and digestion stable protein with an allergenic potency similar to Ara h 2.⁵⁶ Ara h 7 is 35% homologous to Ara h 2, however its allergenic properties have not been further characterised.⁵⁶ Ara h 3 is a peanut glycinin and belongs to the legumin (11s) family of seed storage proteins.⁵⁷ The non-specific lipid transfer protein (LTP) allergen (Arah9) form another family of the prolamin superfamily. They are involved in stabilisation of membranes, cell wall organisation, signal transduction and resistance to biotic and abiotic stress.⁵⁸ They are associated often with primary sensitisation to the major peach allergen Prup3. Arah9 has been associated with more severe symptoms and affects patients in the Mediterranean basin.^{59,60} Other minor allergens in peanut include those associated with pollen sensitisation such as the Betv1 homologue of peanut (Arah8), profilin (Arah5) and the oil-body associated allergens (Arah 10 and 11). In contrast, these, more minor food allergens, including Betv1 homologues and profilins are relatively unstable when exposed to digestive enzymes and thermal processing.

Importance of peanut proteins in peanut allergy

The pattern of binding to peanut proteins varies geographically probably due to different environmental exposures. In the US and UK populations more than 90% have specific IgE to Ara h 1 and Ara h 2 and 45-95% have specific IgE to Ara h 3.⁶¹ In one study comparing peanut allergic patients from Spain, the US and Sweden, differences were found in peanut allergen-binding patterns, the severity of symptoms and the timing of onset of peanut allergy.⁶² Ara h 1, 2 and 3 were found to be the main elicitors of allergic reactions in the USA and often associated with severe symptoms. These allergens were less frequently recognised by the Spanish population who were more often sensitised to the lipid transfer protein. Swedish patients detected Ara h 1 and Ara h 3 more frequently than Spanish patients but had the highest sensitisation rate to Ara h 8, a major cross-reactive homologue of the major birch pollen Bet v 1. In a study examining peanut allergic subjects from across 11 European countries who were sensitised to Ara h 1, Ara h 2 and Ara h 3 since childhood Ara h 2 was identified as the sole major allergen. Ara h 8 and Ara h 9 were identified as the major allergens for Central/Western and Southern Europeans respectively.⁶³ In a study of peanut allergic patients from the Netherlands the most frequently recognised allergen was Ara h 2.⁶⁴ Kukkonen et al demonstrated that co-sensitisation to Ara h 2 and Ara h 6 was associated with severe reactions distinguishing severe allergy from mild symptoms.⁶⁵ In a study of children from the Swedish BAMSE birth cohort children sensitised to both peanut and birch pollen were less likely to report symptoms to peanut than children sensitised to peanut to peanut but not to birch pollen at aged 8.⁶⁶ Sensitisation to peanut oleosins has been associated with severe systemic reactions.⁶⁷

Purification of natural peanut allergens

Purification of natural allergens from peanut may be difficult. Food allergens undergo modification during food processing which are not present in recombinant allergens. Peanuts are consumed after they have been modified through cooking methods such as boiling roasting or frying which can alter the physiochemical properties of the allergens by changing their allergenicity or IgE binding capacity. Peanut allergens make up 22-30% of the total protein in peanut seeds.⁶⁸ Sixteen proteins belonging to 7 protein families are at present classified as allergens. The peanut seed storage allergens Ara h 1 and Ara h 2 make up 12-16% and 5.9-9.2% of the total peanut protein content respectively.⁶⁴

Effects of thermal processing on peanut allergens

Peanuts are eaten in various cooked forms according to culinary tradition. These differing preparation methods seem to have an impact on peanut allergy prevalence. For example boiling a peanut can reduce its allergenicity by reducing the IgE binding capacity of Ara h1, Ara h2 and Ara h3 compared to roasting and countries employing this cooking methods, for example China, seem to have lower incidences of allergy.⁶⁹ The process of roasting a peanut was shown to increase peanut allergenicity and the IgE binding capacity of allergens possibly by glycosylation of protein residues also known as the Maillard reaction.⁷⁰ Frying of peanuts but not boiling or roasting is known to alter the secondary structure of Ara h 2 by decreasing the molecule's content of α helices and increasing its β sheets thereby altering Ara h 2 epitopes and reducing its allergenicity.⁷¹

Diagnosis of peanut allergy

A key component in the diagnostic evaluation of peanut allergy is history taking. It is the role of the physician to correctly identify the potential allergen, timing of symptoms in relation to exposure to the allergen, amount ingested and any comorbid conditions. Once the history has been established then the physician may move to diagnostic tests. IgE mediated food allergy is associated with the presence of allergen-specific IgE antibodies which can be measured in the serum or can be shown by a positive skin-prick test (SPT) to allergen in which a small amount of allergen is introduced into the epidermis using a pinprick and the ensuing wheal and flare response is measured. However sensitisation, the presence of antigen-specific IgE antibodies, does not automatically equate to clinical allergy and it is not sufficient to solely demonstrate the presence of allergic antibodies when diagnosing an allergy. One study which examined 2848 infants found the prevalence of sensitisation to peanut to be 8.9% however when the individuals were challenged the prevalence of challenge proven peanut allergy in sensitised individuals was much lower, 3%.¹⁸ Another study by Nicolau estimated the prevalence of sensitisation to be 11.8% and the prevalence of clinical peanut allergy among sensitised subjects as 22.4%.⁷² Threshold levels have been identified for levels of peanut IgE measured by Immucap (>15 kU/L) that are 95% predictive of clinical reactivity⁷³ although differences in 95% predictive values have been reported across different study populations.^{74,75} Peanut is composed of many proteins (components). Allergen components can either be produced biotechnologically in a recombinant fashion or purified from their original sources. Diagnosis of allergy to peanut is enhanced by measurement of IgE to specific components and may potentially reduce the need for oral food challenges.⁷⁵ For example IgE against the major component Ara h2 has a greater specificity for peanut than IgE

against whole peanut.⁷⁶ It has been shown that sIgE to Ara h 2 could be used as a good predictor of suspected peanut allergy for both children and adults among allergic populations of several geographic regions.⁷⁷ Furthermore in a study by Flinterman et al IgE reactivity to Ara h 2 remained stable over time within an individual.⁷⁸ Codreanu et al showed that a cut off of Ara h 2 > 0.23 would be optimal to separate peanut allergic patients from those who are tolerant.⁷⁹ In contrast studies performed with the peanut allergens Ara h 1 and Ara h 3 showed that the predictive diagnostic value of the their sIgE was low and depended on the geographical area of origin of the study population.^{63,77} In other studies sensitisation to multiple allergens is indicative of more severe reactions than sensitisation to only one of the peanut components.⁸⁰ There are also significant geographical variations in the pattern of binding to peanut proteins.⁵⁹

Oral Food Challenges

Oral challenge testing can be undertaken. This is when the food in question for example, peanut, is introduced in gradually increasing amounts in a controlled environment until either a specific goal dose is achieved or there are objective symptoms of a clinical reaction.

Typically in a clinical setting this is undertaken for 3 main reasons.

1. To establish a firm diagnosis when the diagnosis remains unproven through history, skin prick tests and or elimination diets.
2. If a patient has significant eczema or gastrointestinal disease and specific IgE levels are not in the diagnostic range and there is an unclear response to an elimination diet.
3. To determine if a patient with a known food allergy has developed a tolerance to food.

In a research setting food challenges are undertaken for 3 main reasons:

1. To evaluate the accuracy of existing diagnostic methods such as SPT/ allergen specific serum IgE/patch tests.
2. To establish threshold doses for specific allergens such as peanut
3. To clarify the effect of food processing on allergenicity.

Methods of performing challenges include open challenges, single blind challenges and the double-blind placebo-controlled food challenges (DBPCFC). In an open challenge the food is administered without blinding or use of a placebo. However this may introduce bias on the part of the clinician administering the challenge and the observer. This can result in false

positive challenge results with even up to 30% false positives being reported in some studies.

⁸¹ This challenge is of use when there is high probability that the outcome will be negative. In a single blind challenge the patient is blinded to the challenge material whereas the observer is not. In a double-blind placebo-controlled challenge there are two separate visits to the procedure. On one day the patient receives the food containing the active challenge and on the other day the patient will receive the placebo. Both the investigator and patient are blinded to the order. Although regarded as the 'gold standard' the DBPCFC is not perfect and false positive and false negative rates have been estimated to be between 1 and 3%.⁸²

Nonetheless a food challenge is a very useful tool and one study which examined the impact of food challenges on the quality of life of peanut and tree nut allergy individuals and their caregivers found that food challenges are associated with an improved food-related quality of life in the months following a challenge in patients regardless of whether the challenge outcome was a positive or negative reaction.⁸³ It is therefore clear that having a food allergy has a major impact on the quality of life of an individual. Due care, however must be taken when performing a food challenge as they carry inherent risk. In one study 28% of OFC resulted in systemic and potentially life threatening reactions.⁸⁴

Basophil Activation Test

Basophil activation test is a flow-cytometry based assay that assesses changes in the expression of activation markers on the surface of basophils following antigen stimulation.⁸⁵ As it is a functional assay it uses live basophils in whole bloods to detect the ability of IgE to mediate activation of basophils after stimulation with allergen. It may potentially more closely resemble the clinical phenotype of patients rather than methods which merely detect specific IgE binding to allergen as it tests IgE function which depends not only on the allergen-sIgE levels but also on IgE epitope specificity, affinity and clonality.⁸⁶ The basophils of allergic patients display a dose-dependent expression of activation markers on response to allergen challenge whereas sensitised-tolerant patients do not express or have a much lower expression of activation markers after stimulation with allergen.⁸⁷ Compared to SPT and serum specific IgE it has enhanced specificity with conserved sensitivity. For example in the case of peanut the BAT showed 98% sensitivity and 96% specificity to diagnose peanut allergy. Its utility appears best in cases where conventional allergy tests have failed to diagnose allergy and provocation tests are to be considered.⁸⁸

Quality of life and peanut allergy

Quality of life (QOL) refers to an individual's subjective perception of his or her position in life.⁸⁹ Quality of life is most often assessed by patient report in the form of questionnaires. A food allergy can impact on someone's quality of life in a variety of ways. The need for continuous allergen avoidance and the constant threat of anaphylaxis can cause significant stress and anxiety. Early work which used general health related QOL scales (HQOL) to examine QOL in food allergic patients found that QOL in children was significantly impaired relative to both healthy children¹¹ and children with chronic diseases.^{90,91} Food restriction can also place a significant burden on an individual. A study of peanut and tree nut allergic children found that quality of life was better amongst children who reported eating foods with the 'may contain' label versus patients who reportedly avoided foods with the 'may contain' label.¹¹ Food allergies also have a wider effect for example on the sufferer's caregivers. A study performed by Bollinger et al asked the caregivers of children what kind of impact their child's allergy had on their family. Half of the sample reported that their child's allergy significantly affected their family's social activities including their child playing at a friend's house, attending birthday parties and sleepovers, going out to eat and parental relationships.⁹²

Furthermore travel is another social activity which poses risk to a food allergic individual and restrictions on travel undoubtedly impacts a food allergic individual's life. Work performed by Barnett et al examining food allergic patients' experiences of travel abroad found that foreign travel was considered difficult with inherent risk particularly with regard to airlines or restaurants.⁹³

Although studies on health related quality of life in food allergic children and their caregivers are plentiful, studies on adults are scarce and much more research is required in this area.

Literature Review

Part 2: Thresholds

The Scope of the Problem

As mentioned, presently there is no therapy available in clinical practice for the treatment of peanut allergy. Thus the current strategy in managing this condition is careful avoidance of the allergen and rescue therapy in the event of accidental exposure. Allergens in foods present a risk to allergic individuals. To help food allergic consumers practice safe allergen avoidance, European Legislation mandated that the presence of 14 allergens, deliberately added as ingredients, must be declared on prepacked foods. In December 2014 this was extended to non-prepacked foods and foods eaten outside the home. There were also changes to the way in which the allergenic foods were labelled. Allergens now have to be highlighted in bold and located in a single place i.e. in the ingredients list. However an additional type of labelling exists: precautionary allergen labelling (PAL). This type of labelling is aimed at notifying allergic consumers about allergens which are not deliberately added as ingredients but may be there by chance, for example by contamination during processing methods. With an increasingly complex food manufacturing process, often equipment is shared and several different types of food are processed using the same production line. This PAL often takes the form of warnings such as 'May contain peanut', 'Contains nuts', 'Made in factory where nuts are processed' etc. Unfortunately there is no legislation that governs the use of these advisory labels and furthermore these advisory labels are voluntary. As a result, these labels often send mixed messages to consumers making it difficult for them to make rational decisions about food choices.

The problem with Precautionary Allergen Labelling

PALs are present to try and convey the risk of reaction that a certain food poses. Labels however are very inconsistent making risk assessment difficult. Currently, due to fears over litigation with regards to accidental exposure to food allergens, advisory labels seem to be becoming increasingly common. Food manufacturers take a very risk averse approach to PALs. Patients mistakenly believe that a food with a label which reads 'May contain nuts' poses a greater risk than one which is labelled 'May contain traces'.⁹⁴ However studies have demonstrated that there is often no relationship between the wording that is used on food labels and the amount of allergen that that food actually contains.¹² This inconsistent labelling

practice leads allergic consumers to become distrustful of labels leading them to act in one of two ways: either ignoring these advisory statements completely thereby placing themselves at risk or by avoiding these foods and thus narrowing their food choices considerably. The latter approach may have significant adverse consequences on their nutritional status.⁹⁵ Food allergic consumers need to be able to trust the food label. There should be stricter regulation and legislation in place which governs when to use PAL and some standardisation of these labels. Regulation on when to use PAL could be based on, for example, reference doses for allergens such as peanut which have been derived from the distribution of individual threshold doses in the allergic population. These can be used to determine action levels below which PAL will not be required as ideally PAL should only be used when food manufacturers cannot provide a guarantee, to a defined degree, the absence of unintended allergens to a level that would be likely to be harmful to food allergic consumers.⁹⁶ The absence of the PAL should imply a clear level of agreed safety. Consumers need to be well educated about the process of allergen risk assessment to enable them to trust in food manufacturing and labelling practices. How can this problem therefore be addressed?

Establishing thresholds: The theory

Food policy makers are tasked with assessing the hazard posed by allergenic foods. To do this, risk assessors need information about the response characteristics of the at-risk population and the size of the population at risk. In an ideal scenario it should be possible for risk assessors to calculate the number of reactions that would occur for any given level of residual allergen in a food product if allergic individuals consumed that food. Thus data are required on the levels of allergen which may potentially pose a small risk to most members of the allergic population. There is cause to believe that there is a level of peanut allergen that exists below which no member of the allergic population would react. Indeed in a review on existing threshold data for peanut, Taylor et al concluded that ‘thresholds for common allergenic foods are finite, measurable and above zero.’⁹⁷ Indeed a ‘threshold’ is defined as ‘a limit below which a stimulus causes no reaction’. In the field of toxicology scientists use thresholds to determine the harmful effects of chemicals and pollutants. In this area, a threshold is defined as a dose at or below which a response is not seen in an experimental setting. Techniques on modelling thresholds in toxicology studies have been transposed to modelling thresholds in food allergy. In food allergy a threshold is defined as the amount of protein which evokes an allergic reaction. An individual’s elicitation threshold to an allergen is thought to lie between the No Observed Adverse Effect Level (NOAEL), the highest dose

that will not produce an adverse effect in that person and the Lowest Observed Adverse Effect Level (LOAEL). Data on individuals' thresholds can be obtained through various types of clinical study including diagnostic challenges, threshold finding trials and immunotherapy studies. Data from these studies show that the eliciting dose or LOAEL for peanut allergic individuals can range from a tenth of a milligram to many grams.⁹⁸

Thresholds exist at both an individual and population level. It would be logical to believe that in an allergic population the lower the dose, the milder the symptoms and the lower the proportion of reactive individuals. However there is little data on the proportion of allergic individuals reacting to a given dose as well as limited information on how severity relates to the dose threshold for any given individual. Furthermore it has also been reported in some very sensitive individuals that systemic reactions have resulted from exposure to micrograms of food.⁹⁹

Therefore risk managers can utilise the data on NOAELs and LOAELs gleaned from these studies to examine the distribution of clinical minimum eliciting doses. This useful statistical approach allows inferences to be made about reaction rates to doses out with the experimental range; an advantageous approach given the restriction on being able to test all individuals in the allergic population. From a public health perspective, the optimal outcome would be to define a population threshold where all members of the allergic population are protected. However this 'zero risk' approach unfortunately is not practicable and it is not possible to test the reaction threshold of every member of an allergic population. A more realistic and achievable aim, therefore, is to try and uncover an amount of protein that is unlikely to cause serious adverse effects in the majority of the population at risk. Thus the population threshold can be redefined as the largest amount of allergenic food which will not cause a reaction when tested in a defined proportion of allergic individuals. The Eliciting Dose_x approach is often used and is pragmatic. It refers to the amount of allergen that is predicted to produce a reaction in a defined proportion of the allergic population. For example the ED₁₀, which is commonly referred to in threshold studies, is the dose which will elicit a dose in 10% of the population. Eliciting doses are used to model reference doses which are essentially an index of safe exposure. The United States Environmental Protection Agency defines a reference dose as an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

Types of study

The eliciting dose of peanut varies by several orders of magnitude in the allergic population (0.5mg to 8000-10000mg of whole peanut) ⁹⁸ and this is possibly due to the diversity of studies that the data are derived from. These studies vary in their study entry criteria, dosing regime, dosing interval and food matrix (discussed below).

There have been reports of patients severely reacting to trace amounts of allergen or reacting to peanut via the inhaled route. These often take the form of case reports or case series and do not provide quantitative information. Diagnostic double blind placebo are also a source of threshold data. However it should be noted that these studies were not designed specifically for the purpose of ascertaining allergen thresholds. Data from these studies are often obtained retrospectively and from studies which vary widely in their protocols. In the late 1990s, studies designed specifically to establish allergen thresholds came into fruition. In these studies, investigators aim to establish thresholds by starting at low enough doses of allergen to capture low dose reactors, allow a sufficient dosage interval to allow symptoms to evolve and have strict criteria for stopping the challenge when the reactive threshold is established. These provide the most reliable data on threshold. Lastly immunotherapy studies can give data on thresholds.¹⁰⁰ These studies typically measure reactive threshold before and after therapy and thus provide information on no adverse effect levels (NOAELS) and lowest-observed adverse effect levels (LOAELS).

How design affects the outcome of threshold studies

Procedure

It is critical that threshold finding studies have a low enough starting dose to establish NOAELs and LOAELs. Not having a low enough starting dose runs the risk of having first dose reactors which then renders it impossible to establish a NOAEL in these individuals. Additionally it is important that top dose is high enough to capture high dose reactors. A top dose which is too low runs the risk of false negative results by failure of reaching that subject's high dose threshold and hence a LOAEL. This runs the risk of providing false reassurance to that individual following a non-reactive challenge. In threshold studies placebos play an important role. They enable to symptoms to be better interpreted and help to confirm that during active challenges, symptoms are attributable to the allergen itself rather than anxiety or psychosomatisation. In some studies, placebo doses are interspersed with active doses while other studies have placebo and active arms on separate days. Lastly, a carefully thought out dosing interval is essential. Doses are often administered at 30 minute

intervals to allow assimilation of the peanut protein. However it is likely that the speed of absorption by the gastrointestinal tract will vary across individuals and therefore it is difficult to fully ascertain whether an individual is reacting to a previous cumulative dose or the one recently administered. Thus threshold doses should be reported both as cumulative and discrete entities. Solely using a discrete dose may also result in a more conservative estimate of threshold at a population level. Although it is preferable to leave a fairly long interval to enable symptoms to fully evolve at each dose it is also important to ensure that the interval is short enough to allow top doses to be reached in patients who tolerate greater amounts. There have been recent calls however by Blumchen et al check to extend the dosing interval possible to 2 hours as reactions have been reported at up to 58 minutes post dose.¹⁰¹

Scoring of reactions and stopping criteria can affect any threshold estimate. Some studies have used subjective symptoms such as itch or abdominal pain to stop challenges whereas others use objective criteria. It has been reported that the eliciting dose for subjective symptoms lies below the eliciting dose for objective symptoms sometimes by several doses.¹⁰² This can of course have implications when threshold data from different studies is collated to obtain a common threshold.

Participant inclusion

In statistical modelling, the aim is to extrapolate from the tested population to the allergic population as a whole in order that predictions may be made about the numbers of subjects who may potentially react to doses outside the experimental range. Participants taking part in threshold studies need to reflect the allergic population at large. Volunteers for clinical studies are often highly motivated individuals who are often very cognizant of their allergy and its management. They represent only a subgroup of the at risk population. Some studies select patients from a clinic environment on the basis of their history and results of serum and skin prick IgE tests. This can lead to a highly selected population. Selection from clinic also has the potential to miss patients who are potentially less sensitive, have milder forms of allergy and do not feel the need to seek out specialist help. Failure to include this section of the population may result in more conservative estimates of threshold. Further, some studies for safety reasons actively exclude patients with histories of anaphylaxis or life threatening symptoms. There is a worry that these individuals represent the more sensitive end of the spectrum however clinicians have presented data suggesting that low dose reactivity is not synonymous with the ability to experience a reaction at a low dose.¹⁰³ Care must be taken

therefore when designing a study to recruit patients from as unselected a population as is possible.

Challenge materials

The method of delivery of the allergen is important when considering threshold with respect to both the nature of the allergen (preparation) under investigation and the vehicle in which it is delivered (matrix). Peanut can be processed and eaten in many forms including raw, fried, boiled or roasted and is often eaten as a compound, for example, in chocolate or in a sauce such as satay. For safety reasons, challenges should use most allergenic form of the food. In the case of peanut this is often the roasted form. Work on peanut has shown that roasting increases their allergenicity compared with other cooking methods such as boiling or frying. The roasting process results in the Maillard reaction which results in the glycosylation of amino groups to form more stable compounds.¹⁰⁴ This process results in higher extractable levels of Arah1¹⁰⁵ and demonstrated that the glycosylated form of Arah1 but not the deglycosylated form can act as a Th2 adjuvant by activating dendritic cells to drive the maturation of Th2 cells demonstrated by the increased T cell production of IL4 and IL13. Other ways that cooking may influence allergenicity include alteration of epitope availability or configuration.⁶⁹

Other food compounds delivered in conjunction with peanut protein can also influence the allergen effect. In a study by Grimshaw et al, it was observed that participants suffered more severe reactions and higher eliciting doses than expected when challenged with a high fat recipe compared to a lower fat recipe.⁴⁰ Possible explanations for this include a delay in onset of taste perception induced by high fat food, delays in stomach emptying induced by fat and concealment of allergenic epitopes by a high fat food matrix. Experimentally, chocolate specifically impairs protein detection in vitro¹⁰⁶ which may account for the relatively increased frequency of fatal outcome after compound foods are consumed compared to foods containing unadulterated allergen.^{25,107}

Also the form of peanut used in clinical studies can vary, for example, peanut flour versus roasted whole peanut. However this may be less significant when allergens are heat stable, as is the case with peanut. Indeed Allen et al, when reviewing data from multiple challenge protocols, noted no differences in ED50 roasted peanut versus peanut flour.¹⁰⁸ Differences in allergen content among peanut cultivars has been noted although these are only minor and remain undetermined in vitro.¹⁰⁹

In order to try and reduce bias in double-blind placebo controlled food challenges the allergen must be masked. The active and placebo materials must be indistinguishable. Often this is achieved by masking the taste of the allergen with a stronger taste or smell. To mask the granularity of peanut, foods of a similar texture such as oatmeal can mask the grainy texture of peanut flour.

Interpretation of an adverse reaction

Allergic reactions may be subjective meaning that they are experienced only by the challenged individual or objective meaning that they are identifiable and verifiable by outside observers. Studies have reported that subjective symptoms occur before objective symptoms and often represent early warning signs of an allergic reaction.¹¹⁰ However data are limited on the temporality and importance of these early warning signs. Moreover, a study by Van Erp et al reported that subjective symptoms (oral symptoms and abdominal complaints) were significantly associated with disagreement among clinical observers.¹¹¹ Therefore there is debate over whether eliciting doses based on subjective symptoms are comparable to eliciting doses based on objective symptoms. Objective symptoms are often perceived as more a reliable indication of an allergic reaction to food.

Statistical dose-distribution

As previously mentioned data on thresholds are derived from 3 main sources: diagnostic challenge studies, threshold studies and immunotherapy trials. Often it is possible to derive individuals' NOAELS and LOAELS from these studies.

In the past, data from published clinical studies have been pooled to increase the sample size when estimating population threshold. Data can be reported in terms of grams of peanut flour, whole peanut or peanut protein. One of the preliminary steps therefore is to standardise the allergen amount from the source such as calculating the mg peanut protein in the challenge material. In the following discussion on existing threshold data in the literature statistical models used include LOAELs, NOAELs and Eliciting Dose (discussed before). Endpoints include both subjective and objective symptoms and eliciting doses are reported both as cumulative and discrete doses. It is generally considered that an individual's elicitation threshold actually lies between the NOAEL and the LOAEL. In statistics when the observation is known to lie within an interval rather than the exact value being known, interval censored survival analysis is used¹¹² to model a dose distribution curve. The data were fitted to parametric models using log normal, log logistic and Weibull to select the

apparent best fits. There is no biological rationale for using one parametric model over another. The output of the dose distribution modelling is a reference dose in mg of total protein from the allergenic food.

Existing population threshold data

As previously mentioned the population threshold is defined as the largest amount of peanut that would not cause an adverse reaction in any individual within the total population of peanut allergic individuals. As it is impossible to test and perform challenges on every member of the entire peanut allergic population, estimates of the population threshold are derived from data on individuals' thresholds which have been obtained from clinical food challenge studies. Data from clinical studies which have examined the same allergenic food can be combined. This is advantageous in that a wider range of study participants of potentially varying sensitivities are included. In addition by merging studies, differences in the food matrix as well as in dose regimes are averaged out.

Two studies by Taylor et al^{98,113} have attempted to establish population thresholds for peanut allergy and contain some of the largest data sets for eliciting doses for individuals. Although several studies prior to these had given a wealth of information on the lowest doses of peanut allergen required to elicit a reaction in peanut allergic individuals namely the LOAEL, many of these studies gave little information on the NOAEL (the highest dose which elicits no reaction in peanut allergic individuals). This made it difficult to estimate population thresholds from these studies.

In 2009, Taylor analysed 12 publications for information on peanut thresholds. These publications were based on a spectrum of clinical studies including diagnostic oral food challenges, immunotherapy studies and threshold studies. From these publications information was available on the NOAELs and LOAELs of 185 peanut allergic subjects. These data were analysed using interval censored survival analysis and probability distribution modelling to obtain a population threshold estimate. In this case the ED₁₀ (the amount of protein required to provoke a reaction in 10% allergic population) was found to be 17.6 mg whole peanut using the log-normal distribution. On further analysis of the data from the three different types of study it was found that the ED₁₀ estimates varied significantly between them (11.9mg for threshold studies, 18mg for diagnostic series and 65.5mg for immunotherapy trials). It was thought that this was possibly due to patient selection biases for

example investigators of immunotherapy studies enrolling less sensitive patients with higher NOAELs and LOAELs.

Because of this discrepancy between different challenge studies, Taylor attempted a further analysis in 2010 examining data on a large clinical dataset from Nancy in France where a consistent challenge protocol was used over 10 years. Clinical records of 286 consecutive patients (adults and children) undergoing oral challenges to peanuts were examined. In this study the ED10 and ED5 were 14.4mg and 7.3mg respectively. Furthermore there had always been concern that previous studies had omitted patients with histories of severe reactions and whether these individuals represented a more sensitive section of the population. However it was found that minimum eliciting dose distributions for patients with histories of more severe reactions did not differ significantly from those patients with histories of milder reactions.

More recently Ballmer-Weber looked at threshold distributions for peanut in the Europrevall birth cohort and found an ED10 value of 11.2mg of whole peanut.¹¹⁴ Zhu et al performed a retrospective analysis of published data from OFC with peanut to assess eliciting doses. From their data they proposed an ED10 of 2.8 mg determined by a log normal distribution which is a much lower ED10 than other studies.¹¹⁵ This is possibly explained by the fact that they included data on LOAELs based subjective symptoms which have not been included in other studies. NOAELs based on subjective symptoms have been shown to be much lower than LOAELs based on objective symptoms. A similarly low ED10 and ED5 was reported by Blom et al 2012 who also took subjective symptoms into account (4.4mg and 1.6mg peanut protein respectively).¹⁰² The opposite was true of a study by Eller et al whose population threshold estimates were much higher than any others reported. Based on their data most of which, comprised peanut thresholds from children, they reported ED10 and ED5 of 133.8mg and 77.0mg whole peanut.¹¹⁶ These high threshold estimates are possibly attributable to the fact that this study used a combination of open challenges and DBPCFC with a relatively high starting dose of peanut. In one of the most recent studies by Allen et al, attempts have been made to derive the ED1. A reference dose based on the ED01 would be expected to protect at least 99% of people allergic to the particular food from any objective reaction. They combined data from 55 studies of clinical oral food challenges and have estimated the ED1 to be 0.2 mg of peanut protein (0.8mg whole peanut).¹⁰⁸ In the remaining 1% patients, the hope is that only mild reactions would occur on exposure to these doses, further work is currently being undertaken using single dose challenges to examine this concept and validate these dose levels.¹¹⁷ Going forward, ED1 and ED5 estimates are preferred by regulatory

authorities as they confer a greater level of protection to patients with food allergy however these estimations require larger dose distribution sets. A summary of threshold estimates to date are shown in Table 1.

Table 1: Summary of threshold estimates derived from studies. All values are shown in mg peanut protein (equivalent to 25% of whole peanut).

Group	Study Type	ED10	ED5	ED1
Allen (2014)	Mixed			0.29
Ballmer-Weber (2015)	Threshold	2.80		
Blom (2012)	Threshold	4.42	1.56	0.15
Blumchen (2014)	Threshold	4.10	1.95	
Eller (2011)	Threshold	32.90	18.90	
Klemans (2015)	Threshold	10.80	5.08	
Taylor (2009)	Mixed	4.40		
Taylor (2010)	Diagnostic	3.60	1.83	
Zhu (2015)	Mixed	0.70		

Eliciting doses in relation to severity

When food policy makers are designing reference doses it is useful to them to determine the relationship of reaction severity to the threshold dose. So far data is limited in this regard. Several studies have failed to find a correlation between eliciting dose and severity during challenge.^{101,118} In one study, however, it was observed that peanut allergic adults with moderate to severe reactions during DBPCFC had significantly lower threshold doses compared with those who had mild reactions. It was also shown that a low ED in challenge was correlated to severity of previous reactions.¹¹⁹ However this observation could not be reproduced in other studies involving children.^{120,121} Interestingly a recent study showed that patients with severe reactions to peanut had statistically significantly higher MEDs than patients with mild or moderate reactions.¹¹⁵

Other factors relating to threshold

There has been a great deal of interest recently looking into whether an individual's reaction threshold can be predicted using biochemical or immunological markers or factors related to an individual's history. Blumchen et al found that there was an inverse correlation with the eliciting dose at challenge and the number of accidental reactions and the worst accidental

reaction in the patient's history. Furthermore they found that increasing levels of specific IgE levels to peanut and Arah2, increasing SPT responses and basophil activation and Th2 cytokine production by PMBC (peripheral blood mononuclear cells) were significantly associated with eliciting dose.¹⁰¹ Conversely another study found that there was no correlation between SPT reactivity and level of specific IgE to eliciting dose.¹¹⁸ It was observed in the same study, however, that the DBPCFC seemed to be representative of real life with individuals who had reacted to hidden traces in their history having a lower ED. In terms of associated atopic conditions and their bearing on threshold of reaction, studies have generally agreed that the presence of asthma does not affect the eliciting dose.¹²² One study did find that the presence of atopic dermatitis was associated with higher eliciting doses in children. This is possibly because the presence of atopic dermatitis may mask early symptoms such as itch or urticarial which may occur with lower doses of peanut.¹²⁰ Recent work by Santos et al found that patients with a higher cumulative peanut threshold dose had a higher ratio of peanut-specific IgG4 (a blocking antibody) to IgE. This suggests that IgG4 may be competing with IgE for binding with allergen and blocking its effect.¹²³

Threshold differences between adults and children

Many previous threshold and diagnostic food challenge studies involve children rather than adults. Indeed a large retrospective analysis by Zhu of several published threshold studies found that 67% studies worldwide were based on children.¹¹⁵ It is disputed whether age has an effect on eliciting dose with some studies claiming that increasing age is inversely correlated with eliciting doses¹²⁰ while others have found no effect of age on eliciting dose.^{101,108,122} In recent work by Klemans et al the differences between peanut threshold distribution curves for adults and children were studied. The threshold distribution curves for both objective and subjective symptoms were significantly different between adults and children. In this sample they observed that given a certain dose increase a lower proportion of the adult population reacts to the following dose with objective symptoms compared to the paediatric population suggesting that children react to lower doses with objective symptoms. However overall ED5 and ED10 for objective symptoms were comparable between adults and children. Subjective ED5 and ED10 for adults were significantly lower compared to children.¹²⁴ This may be related to the fact that it may be more difficult for children to note or report subjective symptoms or for doctors to recognise them.

How threshold doses can be translated into clinical practice

The establishment of population thresholds and reference doses would permit public health agencies and the food industry to set limits on the use of precautionary labelling. The establishment of a definite level of peanut allergen which is known to be harmful to a proportion of the population would allow food manufacturers to aim to ensure that their foods remain free of this level of contaminant. Furthermore improvements to labelling can be made. Partly this may involve removing precautionary labels where they are not needed which instilling confidence in peanut allergic consumers. Also establishment of a reliable labelling system free of conflicting advice, informed by evidence would improve safety and reduce overuse. The VITAL initiative, developed in Australia in 2007, was the first formal attempt to establish an evidence based labelling system. The VITAL program developed a grid in which action levels (in parts per million) were defined for major allergenic foods.¹²⁵ It also took into account consumption levels of the food and whether a food contained a dose of the allergenic food above the reference dose, for example, foods typically consumed in large amounts will contain large amounts of allergen compared to foods which are eaten in small amounts such as a condiment or garnish. At the time that this system was instituted limited data existed on minimal eliciting doses and therefore a 10 fold uncertainty factor was applied by VITAL to ensure that sufficiently conservative action levels were promoted. In light of updated data and new knowledge on thresholds this guidance was updated to VITAL 2.0.¹⁰⁸

More recently the ED05 has been established for peanut (1.5mg peanut protein, 6mg whole peanut). Hourihane sought to validate this predicted ED05 by challenging peanut allergic patients with a single dose challenge. Three hundred and seventy eight children were given a dose of 1.5mg peanut protein. Most children experienced no reaction following this dose (65%). Only 2.1% met the criteria for an objective reaction and no child experienced more than a mild reaction.¹¹⁷ In practice this single dose challenge protocol may be used to identify the most dose sensitive members of the allergic population not otherwise identifiable by using standard skin prick tests or specific IgE levels.

Threshold levels across different foods

A few studies have attempted to compare threshold dose distributions across different allergenic foods. Blom et al compared egg, milk, hazelnut and peanut thresholds in a retrospective study of children who underwent DBPCFC.¹⁰² They found that the dose response within the allergic population for the different foods was not equally distributed.

Hazelnut allergic patients reacted to the lowest doses of allergen. They also observed that the dose range to which the peanut allergic population was reacting was smaller and at higher doses the population with peanut allergy was more likely to respond. They found that at a dose of 67mg peanut protein 50% of the allergic population had reacted whereas at the same dose of other allergens such as egg and milk only 35% of the equivalent allergic populations had responded.

This contrasts with Eller et al who found the threshold distributions to be broadly similar amongst hazelnut, milk and peanut.¹¹⁶ Again this different finding may be due to differences in the challenge vehicle and study design.

Literature Review

Part 3: Co-factors

It appears that in an allergic reaction there is not a simple linear relationship between allergen exposure and reaction in a sensitised individual. One study of children who underwent two peanut challenges at an interval showed that almost all children reacted at a different severity score or another dose at the repeat challenge.¹²⁶ Anecdotally, patients report in clinic that their allergic reaction seems to change in different situations. Therefore it seems that there perhaps may be other factors responsible for modulating an allergic reaction. When considering these ‘other’ factors, two groups are thought to exist: host (intrinsic factors) factors and event (extrinsic) factors. Host factors are factors which specifically pertain to an individual such as atopic disease, age, previous reactions, comorbidities, physiological compensatory capability and gastrointestinal absorption. Event factors are external factors which can vary with time or certain situations and include such things as exercise, psychological stress, alcohol, intercurrent infections and use of medications such as non steroidal. It has been suggested that co-factors may in some way be responsible for augmenting allergic reactions. Indeed in peanut immunotherapy studies it has been reported that patients seemed to lose tolerance to peanut doses during both the up dosing and maintenance periods when they took the doses close to periods of exercise or when they were tired.¹²⁷ Co-factors have been identified in up to 30% of anaphylactic reactions in adults¹²⁸ with a variety of co-factors being implicated (Table 2). In the presence of these factors, allergic reactions may be elicited at lower doses or may be more severe or life threatening. However, underlying mechanisms have so far yet to be elucidated.

Table 2: Prevalence estimates of co-factor involvement in anaphylactic reactions.

Adapted from Wolbing et al.¹²⁸

Co-factor	Prevalence estimate
Exercise	1-15.9%
Alcohol	1-15.2%
Infection	1.3-11%
NSAID	6.1-9%
Mental stress	8%
Other e.g. menstruation	12.1%

Exercise as a co-factor

Physical exercise is the best known and best studied co-factor in anaphylaxis. There are few data in existence on how physical exercise modifies an allergic reaction to peanut in an individual who is known to be peanut allergic. Instead the clinically distinct condition, Food Dependent Exercise-Induced Anaphylaxis, serves as a model condition for understanding how exercise may play a role. In this disorder, the triggering food is tolerated under normal conditions but when the food is consumed in association with physical exercise, it is no longer tolerated and an allergic reaction usually ensues.¹²⁹ The intensity of exercise required to induce an allergic reaction is variable with reports of this condition occurring with both strenuous exercise and even very light physical activity such as walking or gardening.

Food Dependent Exercise Induced Anaphylaxis

The most common form of this condition is 'Wheat-Dependent Exercise-Induced Anaphylaxis' (WDEIA). Two possible mechanisms for the underlying pathophysiology have been proposed:

1. The bioavailability and distribution of certain allergens may be increased by exercise
2. The threshold for activation of mast cells and basophils may be decreased by exercise

Matsuo and colleagues investigated individuals with WDEIA. They observed significantly increased levels of immunoreactive gliadin in the sera of individuals after consumption of wheat combined with physical exercise compared with consumption of wheat without the application of physical exercise. This change was also observed in healthy volunteers.¹³⁰

However it should be noted in this condition that exercise is not always the essential co-factor. Recently, Brockow et al were able to reproduce symptoms in 4 patients with a history of WDEIA by challenging them with gluten, acetylsalicylic acid (ASA) and alcohol without the involvement of exercise.¹³¹

Indeed in the aforementioned study by Matsuo et al it was demonstrated that acetylsalicylic acid (ASA) increased the amount of serum gliadin by five times after wheat consumption.¹³² The exact mechanism of how aspirin exerts its effect is yet to be fully elucidated however various theories have been suggested. Firstly it has been suggested that aspirin disrupts the integrity of cellular tight junctions thereby increasing gastric permeability.¹³³ Secondly ASA

inhibits cyclooxygenase function resulting in decreased prostaglandin and increased leukotriene synthesis. In a study by Inoue et al they found after administering a synthetic analogue of prostaglandin E1 (Misoprostol) which compensated the ASA-induced inhibition of prostaglandin synthesis, that allergen absorption and the outbreak of allergic symptoms induced by ASA in patients with WDEIA may be suppressed.¹³⁴ Lastly ASA has been reported to directly augment the degranulation of mast cells and accelerate histamine release via increased Syk kinase activation.¹³²

Physiological changes during exercise

Increases in gastric permeability

Intestinal uptake of food proteins occurs via two routes across the epithelium: transcellular or paracellular. Diffusion, active transport or endocytosis utilise the transcellular route whereas paracellular transport is characterised by the transport of larger molecules through tight junctions into the interstitial space. These molecules then enter the systemic circulation from the interstitial fluid between the enterocytes.

Physiologically, exercise induces changes conducive to enabling the above reactions to take place. Much work previously has focussed on alterations to intestinal absorption through alterations in blood flow during exercise. These changes were demonstrated elegantly in a study by Van Wijck et al who examined healthy men undertaking 60 minutes of cycling at 70% of maximum work load capacity. They assessed splanchnic hypoperfusion through gastric tonometry, enterocyte damage parameters through serum markers (intestinal fatty acid binding protein (I-FABP) and ileal bile acid binding protein (I-BABP) and intestinal permeability through sugar probes. They found that splanchnic perfusion fell during exercise and that hypoperfusion increased plasma I-FABP, I-BABP and hypoperfusion correlated significantly with small intestinal damage. An increase in small intestinal permeability after exercise was seen which correlated with intestinal injury.¹³⁵ Therefore should allergen absorption be combined with exercise, intuitively it follows that the absorption of large molecules such as intact allergens would be facilitated through a more permeable gastric mucosa, therefore having access to the gut-associated immune system. Pals et al also demonstrated in a group of healthy participants that running at 80% VO₂ max (maximum exercise capacity) increased small intestinal permeability measured through urinary excretion rates of the lactulose to rhamnose ratio compared with rest and lower exercise intensities (40 and 60%).¹³⁶

Blood flow redistribution and mast cell heterogeneity

During exercise blood is redistributed away from visceral organs (kidneys, gastrointestinal system) towards working muscles and organs (skeletal muscle, lungs, heart and skin).

Robson-Ansley et al hypothesised that the sudden redistribution of blood transports the allergen away from the gut to the skin or skeletal muscle where phenotypically different mast cells reside resulting in altered mediator release and an altered disposition for developing anaphylaxis.¹³⁷ Currently however there is no experimental evidence to support or refute this theory.

Changes to plasma osmolality inducing basophil histamine release.

It has been suggested that plasma osmolality may increase marginally during prolonged physical activity. In vitro studies have indicated that alterations in plasma osmolality can increase basophil activation and histamine release.^{138,139} By performing histamine release assays using a range of buffers with increasing osmolality, Barg et al demonstrated on basophils from patients with FDEIA, food allergy and healthy controls that histamine release and basophil activation increased in the patient with FDEIA at 340 nOsm and not in the food allergic or control subjects.¹³⁸ It is possible to hypothesise that physical activity may induce a transient serum hyperosmolality which may trigger increased basophil reactivity and histamine release on exposure to a food allergen.

Other possible proposed mechanisms include the increased release of mediators during exercise which sensitise the calcium channel through which histamine works (TRPV1 receptor) and also local and systemic changes in acid-base balance during acute exercise whereby a cellular-reduced pH promotes mast cell degranulation heightening a propensity towards anaphylaxis.¹⁴⁰

Stress and sleep deprivation as cofactors

Stress has also been described as an augmenting cofactor in allergic reactions. Generalised stress or malaise has been described as a contributory factor in fatal allergic reactions.¹⁴¹ A well-established clinical observation is that stress can exacerbate urticaria and angioedema, particularly non-IgE mediated forms. The mechanism is unknown, but a reduced threshold for mast cell activation is likely.¹⁴² In a recent immunotherapy study it was shown that acquired tolerance to peanut was reversed under the influence of co-factors. Six out of 20 individuals were unable to tolerate a dose that they had previously been able to tolerate if

they had suffered restricted sleep (<6 hours).¹⁴³ Acute sleep loss is a potent stimulus of stress hormones in animals.¹⁴⁴ Rather than subjecting patients to psychological stress, in this study we decided to use sleep restriction as a proxy for stress. Moreover sleep restriction has the benefit of being easily reproducible, measurable and the ensuing tiredness relatively long lasting.

Clinical and experimental studies in human and animal models have shown that sleep loss can lead to impaired immune function. It has been suggested that cellular immune responses can be disrupted by shifting T helper cell activity towards a Th2 cytokine profile. This was demonstrated by Axelsson et al who investigated partial sleep deprivation for five days and the production of inflammatory cytokines and the Th1/Th2 balance in healthy subjects. They noted a shift towards Th2 activity determined by a decrease in IL-2/IL4 ratio.¹⁴⁵ Sleep loss exerts a significant regulatory influence on peripheral levels of inflammatory mediators of the immune response. Sakami et al also noted this shift in insomniacs. In their study they noted a significantly lower IFN-gamma and ratio of IFNgamma/IL4 than non-insomniac men again suggesting a Th2 dominant profile.¹⁴⁶ In anaphylaxis clinical manifestations arise as a result of mast cell and basophil inflammatory mediators acting on a variety of target organs. In addition to these mediators, several cytokines are released into the milieu including IL4, IL5, IL13, IL16 TNF α and various chemokines. These act on various effector cells propagating the allergic response by cell activation, chemoattraction, IgE production and increased Fc ϵ R1 expression. Changes in cytokine levels have been observed in various studies subjecting individuals to sleep deprivation protocols. In particular, a study of partial sleep deprivation (4 hours) found increased production of TNF α and IL1 β following stimulation of peripheral blood mononuclear cells with Lipopolysaccharide and Polyhydroxyalkanoate respectively.¹⁴⁷

Alcohol as a cofactor

Ethanol, like exercise, can also activate the TRPV1 channel. This can lower the threshold to activation by products of allergic inflammation which act via this ion channel. TRPV1 activation causes neuromediator release including calcitonin gene related peptide which can result in vasodilatation.¹⁴⁸ The vasodilatory response may exacerbate shock in anaphylaxis. Alcohol may also cause gastritis, permitting greater allergen absorption. and augment histamine release.¹⁴⁹

Literature review

Part 4:

Symptoms and severity of peanut allergic reactions

Allergic reactions vary in severity. This variation occurs across individuals in a population but in addition, a patient's own reaction severity can change from one allergen exposure to the next. It seems intuitive that the dose of allergen that a person is exposed to determines the severity of reaction implying that a greater dose of allergen leads to an increase in severity. However severe laryngeal symptoms requiring adrenaline administration have been reported for doses as low as 0.3-1mg of peanut protein.^{119,143} A good understanding of a patient's reaction severity is crucial for clinicians as it can guide them on both acute and long term management of that individual. Furthermore knowledge about reaction severity and symptom progression can enhance safety in oral food challenges by instructing physicians on when to stop a challenge and administer treatment. Unfortunately limited data exist on the evolution of allergic symptoms during challenge. Further the classification of allergic reaction severity is problematic as various grading systems are used to report systemic reactions but none are globally accepted: venom¹⁵⁰, food^{80,151–153}, drugs¹⁵⁴ and adverse reactions to allergen immunotherapy.¹⁵⁵ Furthermore each system has usually been developed based on observations of patients with different types of allergy which may differ in symptom pattern and thus different weights are attributed to different symptoms across the scores. An implication of contrasting classification systems is that comparison of clinical practice becomes problematic. From a research point of view there may be difficulties in assessing the efficacy of treatments (e.g. immunotherapy), interventions (e.g. adrenaline administration) or patient outcomes. Thus further fine grain detail on symptom severity will inevitably lead to the development of a more universal classification system.

Symptoms associated with peanut allergic reactions

In general, symptoms of an allergic reaction usually reflect the route of allergic exposure. Thus in reactions triggered by parenterally administered agents such as intravenous drugs or venom, cardiovascular manifestations such as hypotension usually predominate. In contrast with food allergic reactions, the skin and mucous membranes are involved as these represent the first point of contact between the individual and their environment. In an IgE-mediated reaction to peanut cutaneous manifestations are usually the most common occurring in about 74% patients with an immediate reaction to food.¹⁵⁶ In a series by Perry et al, 253 failed food

challenge reactions were examined and the investigators found that gastrointestinal symptoms also were common occurring in 43% of failed challenges. Oral, upper respiratory and lower respiratory symptoms were less common and occurred in approximately 25% of failed challenges.⁸⁴ Distinct behavioural changes are also very common especially in young children. It is common to see children change suddenly from happy and playful to quiet and frightened with the onset of a reaction. In Food Dependent Exercise Induced Anaphylaxis, symptom patterns may be different often starting with pruritus of the scalp which then becomes generalised followed by respiratory obstruction and occasionally cardiovascular collapse.¹⁵³ Severe and life threatening reactions are rare. Symptoms of food anaphylaxis may develop as soon as 3 minutes after exposure to usually up to 2 hours after the ingestion of a food allergen.¹⁰¹ However the vast majority of food allergic reactions happen within 30 minutes. Biphase reactions occur when symptoms recur after apparent initial resolution. In a systematic review and meta-analysis of 27 observational studies biphasic episodes occurred in 192/4114 (4.7%) patients with anaphylaxis with the range varying between 0.4-23.3%. Patients with hypotension and unknown triggers were at greater risk.¹⁵⁷

Fatal anaphylaxis

Peanut has the propensity to cause fatal allergic reactions. However the exact incidence of fatal anaphylaxis for food allergic people is unknown. In general, the majority of studies suggest that although the overall incidence of anaphylaxis is increasing, there does not seem to be an increase in fatalities from this condition.^{158,159} However there is one recent Australian study which did report a rise in fatal cases.¹⁶⁰ Umasunthar et al estimated the incidence rate of fatal food anaphylaxis based on 10 studies in a food-allergic person as 1.81 (95% CI 0.94, 3.45 range 0.63, 6.68) per million person years.¹⁶¹ In a recent study examining databases from the Office of National Statistics between 1992 and 2012, 124 fatalities were attributed to food-induced anaphylaxis. The triggering food was identified in 95/124 cases (77%). Peanut was responsible in 22% adult cases (>16 years old) and in 16% paediatric cases (<16 years old). The percentage of fatalities caused by peanut and tree nut is higher in US registries compared to UK but this may be due to the data being based on voluntary registries in the US.^{25,26}

The mode of death in anaphylaxis has previously been reviewed.^{41,162,163} The major causes are asphyxia from upper and lower airway (45%), shock (41%), unknown (9%), Disseminated Intravascular Coagulation (3%) and epinephrine overdose (2%).¹⁶²

Risk factors for fatal anaphylaxis have been identified. Delayed or lack of administration of adrenaline has unequivocally been found to be a major contributor to mortality.¹⁶⁴ Pumphrey et al found in a fatal anaphylaxis registry that adrenaline was only administered in 62% of fatal reactions and before arrest in only 14%. However, timely administration of adrenaline was not sufficient in some cases to avert a fatal outcome. In the series of 48 cases reviewed by Pumphrey, 3 patients died despite receiving adrenaline from their own kit at the onset of reaction.¹⁶⁵ Asthma is present as a co-morbid condition in more than 90% of fatal reactions.^{166–168} In particular poor asthma control is linked to fatalities. This may be a feature in younger age groups where medication compliance is potentially an issue. A preponderance for younger age has been shown. Young adults or adolescents are particularly at risk.^{4,25} This may possibly be due to an increased risk-taking behaviour in this age group leading to an increase in co-factor exposure for example sleep deprivation or alcohol. Other contributory factors include a failure to carry their adrenaline autoinjector or even disease denial. Yunginger et al evaluated 13 cases of fatal and near fatal anaphylaxis and also reported that failure to identify the responsible food allergen in the meal was a significant risk factor.¹⁶⁹ In instances such as these there may be concealment of the allergen and increased dose exposure.

Risk multipliers in anaphylaxis

The presence of a known risk factor for anaphylaxis can enable clinicians to instigate strategies to reduce the risk of this potentially fatal condition. The role of co-factors in amplifying allergic reactions has been discussed in the previous chapter. However intrinsic host-level factors have also been proposed which may modify allergic reactions. At an immunological level, Nowak-Wegrzyn¹⁷⁰ reported that individuals who have IgE to specific allergenic epitopes which are more heat stable may have a predisposition to more severe reactions. El-Khouly et al showed in 40 peanut allergic patients that both IgE and IgG avidity to Arah2 showed a weak positive correlation to severity challenge score.¹⁷¹ Underlying cardiovascular disease and cardiovascular medications may influence reaction severity. Nassiri et al showed in human and mouse models that singly beta-blockers and ACE inhibitors can increase the severity of anaphylaxis and this effect was more pronounced if the two drugs were combined. The proposed mechanism is that these drugs can act synergistically to decrease the threshold of mast cell activation.¹⁷² However in a study by Brown et al, although cardiovascular risk and medications had a significant association with age, there was a lack of a correlation with symptom severity.¹⁷³ Possible factors associated

with the absence of significant reactions to peanut in allergic individuals include isolated specific IgE to Arah8, the PR-10 related component and suggests Pollen Food Syndrome rather than primary peanut allergy. These individuals usually demonstrate clinical tolerance on exposure to peanut or mild subjective oral symptoms.¹⁷⁴

Literature review

Part 5:

Diagnosis of an acute allergic reaction: mast cell tryptase

Diagnosing anaphylaxis acutely can sometimes be problematic for acute physicians. This is because the symptoms of anaphylaxis: wheeze, flushing, hypotension and angioedema for example can be presenting features of other, more common conditions. Furthermore an allergic trigger may not always be immediately obvious. As a consequence of this, anaphylaxis is under-diagnosed and under-reported.^{175,176} Diagnosing anaphylaxis is imperative as correct diagnosis allows secondary prevention strategies to be implemented such as allergen avoidance, immunotherapy and provision of adrenaline auto injectors.

When anaphylaxis occurs, numerous mediators and pathways may be activated. However not all are measurable in routine clinical practice. Mast cell tryptase has been reported, in national guidelines, to be the most useful blood test to confirm a diagnosis of anaphylaxis if correctly performed.¹⁷⁷ Tryptase measurement, however, has the potential to be useless if samples are incorrectly timed. Tryptase is released from mast cells, crucial cells which belong to the innate arm of the immune system. Mast cells are present at interfaces between the organism and environment for example skin, bronchi and gut and have many immunoregulatory functions including pathogen clearance to inflammation resolution. Tryptase belongs to the family of trypsin-like serine proteases and is the most abundant mediator in mast cell granules. Tryptase is highly specific for mast cells as only trace amounts <1% are detected in basophils and none in other human cells.¹⁷⁸ During IgE mediated degranulation mast cell granules fuse with the plasma membrane and release dozens of preformed mediators including mature tryptase into the extracellular space.^{179,180} The release of secretory granule contents into the extracellular environment occurs in a matter of minutes. A rise in histamine may be detected in the first 5 minutes, however levels only remain elevated for 30-60 minutes and thus capturing peak levels is difficult as there may be a delay in the patient presenting to hospital. Tryptase, on the other hand, is released from mast cells in a complex protease-proteoglycan complex which diffuses slowly. As a result of this, elevation of serum tryptase concentrations may not be detectable in the first 15 or 30 minutes¹⁸¹ however once in the bloodstream the half-life of serum tryptase is about 2 hours. Thus levels will remain elevated between 30 minutes and 2 hours after the onset of anaphylaxis making this the optimal time to sample.¹⁸² This allows enough time for

degranulated tryptase to reach peripheral blood and short enough to avoid missing elevated serum tryptase due to plasma clearance. This lowers the risk of obtaining a false negative by too early or too late measurements. Therefore due to its relatively long biological half-life and stability in serum stored at -20°C tryptase is a convenient surrogate marker for other pharmacologically active mediators which are released at the same time. Baseline or 'constitutive' mast cell tryptase levels are the result of continuous release of immature inactive alpha and beta tryptase monomers (pro-tryptases) and reflect the number of mast cells. Mature beta tryptase is contained in secretory granules and released only when mast cells degranulate.¹⁸³ Currently there is only one commercially available tryptase test which detects both immature and mature forms of alpha and beta tryptase in body fluids (ImmunoCAP Tryptase, Thermofisher Scientific Diagnostics, Phadia, Sweden).¹⁸⁴ With this current method, according to data obtained from 126 healthy donors, 95% individuals displayed tryptase values of less than 11.4 µg/L and a median value of 3.8 µg/L. It is generally thought that tryptase values are stable in a given individual over time.

The usefulness of mast cell tryptase has been demonstrated widely in studies of venom and general anaesthetic induced anaphylaxis. It is often raised in reactions which are severe and feature hypotension. Other studies however are reticent about its utility. Sala-Cunill studied patients presenting with acute anaphylaxis and found that mast cell tryptase was not raised in 36.3% of cases. In a study by Stone et al, tryptase was elevated in 55% of moderate reactions and in 75% severe reactions.¹⁸⁵ Lin et al prospectively sampled β and total tryptase in 97 adults presenting to A&E and found raised tryptase in only 20/97 (20%) patients.¹⁸⁶ However the sampling time points are not clear from this study nor whether serial samples were taken which could, in part, explain the few elevated readings. However a few of these studies attributed the lack of a rise in tryptase to the fact that they contained a large proportion of food allergic reactions. Previously it has been reported that tryptase levels fail to rise in food allergic reactions. This is largely based on an analysis of 5 samples (1 postmortem, 1 near fatal and 3 with severe food anaphylaxis). Tryptase failed to significantly rise in all cases. Proposed reasons for this include a predominantly basophil mediated rather than mast cell mediated response or in the fatal case that death intervened before a rise could occur. Post mortem samples in general have been reported to be less sensitive and specific as a non-specific tryptase release may occur after death.¹⁸⁷ It is believed that tryptase levels peak at 1-2 hours and therefore a single measurement of tryptase has a low sensitivity as a peak may be missed or may occur within the normal range for the assay. Borer-Reinhold et al used a

relative increase in tryptase compared to the baseline value when investigating patients who developed systemic reaction to stings. They found that a relative tryptase increase to $\geq 135\%$ above the baseline value (relative delta bound) indicated mast cell activation in these patients even below the upper limit of the tryptase assay (11.4ng/l).¹⁸⁸ There are limited data on the application of this principle to food allergic reactions with one study examining tryptase changes in acute allergic reactions to shrimp in shellfish allergic patients. Thirty two patients suffered allergic reactions during challenge, 38% anaphylaxis. They reported that delta-tryptase (peak minus baseline) levels were higher in the anaphylaxis group compared to non-reactive and milder reactions. They identified a cut-off using ROC analysis of $\geq 0.8\mu\text{g/L}$ (83% sensitivity and 93% specificity).¹⁸⁹ Thus use of a ratio (peak related to baseline) is thought to improve sensitivity, specificity and positive and negative likelihood ratios.

Many mediators are generated during anaphylaxis by numerous cell types. Tryptase is only one of these mediators, may not be the predominant one and may only be released in very small amounts. More recently platelet activating factor (PAF) has been identified as an important mediator in anaphylaxis and in an animal model interventions which block PAF prevent fatal anaphylaxis.¹⁹⁰ Vadas et al measured PAF and PAF acetylhydrolase activity in 41 patients with anaphylaxis and 23 control patients and found that serum PAF levels were directly correlated and serum PAF acetylhydrolase activity was inversely correlated with the severity of anaphylaxis.¹⁹¹ In a further study when comparing PAF with histamine and tryptase neither histamine nor tryptase showed as good correlation with severity scores during reaction as did PAF.¹⁹² Prostaglandin determinations are available commercially and can be of value in the diagnosis of anaphylactic events. In a study of patients with systemic mastocytosis who experienced anaphylaxis it was found that anaphylaxis could be diagnosed by a selective excessive release of prostaglandin D2.¹⁹³ With regard to basophil activation it has been demonstrated that BAT may correlate with symptom severity for peanut allergy.¹⁹⁴ However basal basophil responsiveness is known to vary from day to day within an individual and so may not predict reaction severity on a different day.¹⁹⁵ Therefore using a multiple mediator approach i.e. using numerous markers simultaneously may improve the sensitivity of laboratory tests.

CHAPTER 2

Methods

The Thresholds Reactivity and Clinical Evaluation (TRACE) Study upon which this thesis is based was a 52 week multicentre randomised cross over study. One hundred participants were recruited across two centres carrying out the food challenges: Addenbrooke's Hospital, Cambridge and the Royal Brompton Hospital, London, United Kingdom. For this dissertation, only the results from the Cambridge cohort, whom I personally administered peanut challenges to, will be reported. Pilot work was used to determine the feasibility of the study method. This will be described in 'Pilot Work' following description of the main study method.

Setting

All clinical procedures were carried out in the Wellcome Trust/NIHR Clinical Research Facility, Cambridge.

Recruitment and population

Participants were recruited from the general adult peanut-allergic population. A media agency (MWI) was employed to generate a study identity for a website and advertising materials. Participants were recruited through advertisements on London and Cambridge-based Newspapers (Metro and Evening Standard), Facebook, Google Words and Twitter advertising campaigns. Interested participants were directed to a dedicated study website where further information about the trial was available. Through this website, potential participants registered their interest and answered some simple screening questions including: 'Has your worst reaction to peanut only been mouth or lip swelling?', 'Has your allergy been diagnosed by a doctor?', 'Are you able to run on a treadmill for 10 minutes?' and 'Are you available to take part in the study for the next 12 months?'. Registered participants were added automatically to a database including the responses to screening questions. Following this registered eligible participants underwent a brief telephone consultation as a further screening stage.

Study inclusion and exclusion criteria

Inclusion criteria

- Male and female subjects who are 18-45 years of age at the time of study entry

- A diagnosis of peanut allergy as manifested by urticaria, angioedema or respiratory/gastrointestinal tract symptoms, with acute onset of symptoms after ingestion (up to 2h).
- A positive peanut DBPCFC at baseline (Visit 1). This outcome was defined as the onset of objective or significant subjective allergic events after ingestion of peanut protein but not to the placebo.
- Sensitisation to peanut demonstrated by skin prick test, or serum specific IgE

Exclusion criteria

- Oral allergy syndrome to peanut (defined as a clinical history of only oral allergy symptoms on exposure to peanut and principal sensitization to only PR10 homologues of peanut (Ara h 8)
- Monosensitisation to Ara h 9
- History of hypersensitivity to the matrix components used within the challenge material.
- Poorly controlled asthma.
- History of significant and repeated exercise –induced asthma attacks requiring treatment, independent of food ingestion or a drop in FEV1 of >15% during screening Vo2max exercise session
- History of any of the following:
 - Severe anaphylaxis to peanut as defined by hypoxia ($SpO_2 < 92\%$) or hypotension (>30% drop in systolic blood pressure), with or without neurological compromise
 - A previous reaction to peanut that in the opinion of the investigator was life-threatening
 - Mastocytosis

Other exclusion criteria include conditions which would directly impair the participant's ability to undertake the study protocol such as musculoskeletal disorders impairing exercise and shift working impairing the sleep deprivation challenge.

Screening visit

Suitable participants were invited for a screening visit which involved a detailed history, skin prick and blood tests (specific IgE to peanut and Ara h 1, 2, 3, 8 and 9) to determine their allergic status. Each participant underwent a VO2 max test to ascertain their maximum

exercise capacity which was used to determine the exercise intensity for their exercise challenge. Lung function was assessed through spirometry and participants with asthma undertook the Asthma Control Test to determine their asthma control. Pre and post exercise lung function was used to exclude any participants with exercise induced asthma. A fall in FEV1 of greater than 10% following exercise excluded participants from the study.

Informed consent

Each participant received a Participant Information Leaflet and a detailed verbal explanation of the study protocol including the risks and benefits of participation. Potential participants were given the opportunity to ask questions and were provided with sufficient time to make a decision. No clinical procedures were undertaken until informed consent had been obtained. Participants could withdraw at any time without the need for explanation.

Data collection and management

Participant demographic data, details of symptoms during challenge including timing of onset and resolution, threshold reached and treatment administered during challenge was collected on a paper case record form. Data from these forms were then entered by the study team onto a centralised computerised database managed by the University of Manchester.

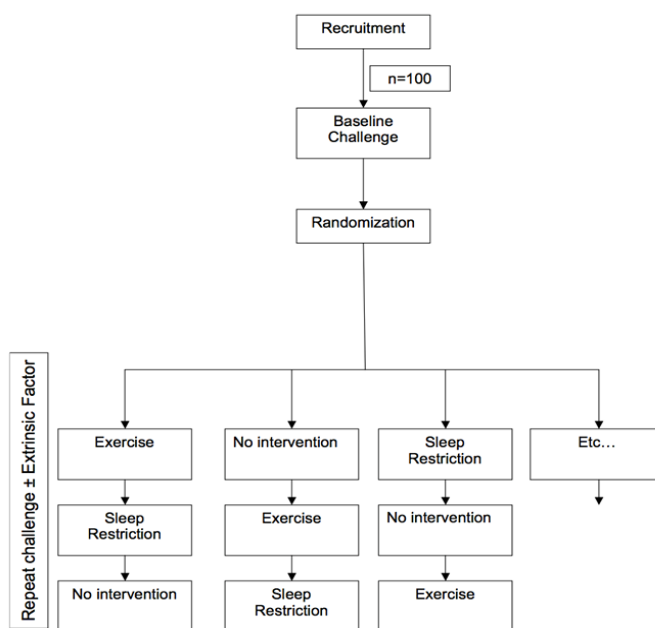
Quality control of the data and data checking was carried out by an independent person at each site who checked 100% of the primary outcome data (cumulative peanut dose reached) and symptoms at the time of onset of reaction.

Study design

Each participant underwent a baseline challenge (to determine their allergic status and initial threshold) followed by a further 3 interventional challenges (exercise, sleep and no intervention) spaced 3 months apart to reduce the possibility of a desensitisation effect produced by repeated peanut challenges (Figure1). The initial baseline challenge was double blind and placebo controlled and took place on 2 separate days: one day active and one day placebo. The order of the two days was randomly assigned and determined by randomisation lists produced by the study statisticians. Both participants and investigators were blinded to the ordering of the challenge days. Following this there was a further randomisation step. There were six allocation arms which varied by the order of the final 3 challenges (exercise/sleep/no intervention). The order of challenges between the six groups was balanced by employing a Latin square design. Participants were randomly assigned to one of

the six arms using a secure online tool with audit trail. The six possible sequences were, ABC/BCA/CAB/ACB/BAC/CBA with each letter representing a different co-factor: A for exercise, B for sleep deprivation and C for no intervention. The strong degree of balance allowed for natural variations in intra-individual threshold over time. Randomisation was stratified by age, centre and the presence of asthma. (Figure 1). The final three challenges were not placebo controlled and participants received the active arm only.

Figure 1: Study design



Oral food challenge procedure

Baseline challenge

A series of eight doses of peanut in the form of peanut flour were prepared. This was incorporated into a masked dessert food carrier developed for a European epidemiological project (Europrevall)¹⁹⁶ and manufactured at the University of Manchester and then distributed to the study centres for reconstitution at the point of use. Microbiological safety and allergen content were confirmed before materials sent out to the clinical centres. The dosing regimen is shown in Table 3. Numerous dosing schedules are currently in use for performing food challenges. Incremental scales vary from 10 fold increases, semi-logarithmic, doubling dose or even smaller increases with the latter associated with schedules aiming to deliver cumulative doses with shorter time intervals between doses (15 minutes). With schedules aimed at delivering discrete doses, intervals are typically longer (30 minutes).

Using lower starting doses and prolonged intervals can increase the likelihood of partial desensitisation and false-negative results.¹⁹⁷ Data from the literature suggests that a starting dose of 3 micrograms should be low enough to provide No Observed Adverse Effect levels (NOAELS, the highest dose known to not induce an objective allergic reaction). However starting at very low doses can make it more difficult to achieve meaningful top doses with acceptable increments in an acceptable period of time. Thus a combination of logarithmic increments (from 3 micrograms to 30mg) followed by semi-logarithmic increments thereafter was used. Usually dosing regimens escalate to high cumulative dose of allergen protein to reduce the risk of a false negative challenge. Sicherer et al reported approximately 5% false-negative challenge results with a top dose of 876mg of protein.¹⁹⁸ In this study, doses were delivered at 30 minute intervals which has been proposed as a suitable interval for the investigation of IgE associated reactions.⁸² However if significant symptoms evolved during the interval, the clinical investigator could increase the interval to 60 minutes. Longer time intervals however lengthen the challenge procedure and decrease the chance of accumulating high doses which may result in more severe reactions.¹⁹⁹ The doses were given until the participant was judged to have developed objective signs of an allergic reaction and thus have reached their clinical threshold. Allergic reactions were treated appropriately and all treatments and their effect were recorded. On a separate day the participant underwent a further challenge where all doses administered were placebo. The placebo dessert matrix matched the active dessert having previously been subjected to blinding tests at the University of Manchester. Placebo challenges were carried out under similar conditions. Both active and placebo challenges occurred in a random order for each participant and were spaced a week apart.

Table 3: Dose regimen

Dose Number	1	2	3	4	5	6	7	8
Amount of peanut protein	3µg	30µg	300µg	3mg	30mg	100mg	300mg	1g

Criteria for scoring symptoms and stopping food challenge

In this study the PRACTALL criteria proposed by Sampson et al⁸² were adapted following our pilot challenge experience (described in ‘Pilot Work’) (Appendix 1). Participants were

deemed to have reached their threshold, and the challenge stopped if they developed three concurrent yellow symptoms within one organ system or across different organ systems or 1 red symptom in any organ system. If the participant was almost at their threshold (e.g. 2 yellow symptoms) and the investigator was concerned about escalating to the next dose level for fear of inducing severe symptoms then the dose could be repeated.

The colour coded symptom grading system was used as follows:

Green (mild) symptoms were not an indication to alter dosing.

Yellow (moderate) symptoms if present singly would be an indication for the investigator to proceed with caution. If three yellow symptoms were present concurrently within the same organ or across different organ systems then this was an indication to stop.

Red (severe) symptoms if present singly was an immediate indication to stop.

The challenge could also be stopped at the investigator's discretion if they believed that continuing the challenge would place the participant at risk or also if the participant did not wish to continue.

Any extra symptoms which did not form part of the stopping criteria were recorded on the case record form as 'Free text symptoms'.

Exercise challenge

On the challenge day the participant was admitted to the ward on the day of the exercise challenge. Participants were given each dose followed 5 minutes later by a 10 minute bout of exercise at 85% VO₂ max on a static bike. Heart rate was measured throughout the challenge using an Actiheart monitor to ensure that they achieved their target heart rate. The participant was allowed to drink water but not able to eat any food apart from the challenge meal.

Sleep challenge

Participants received a peanut challenge after being sleep deprived. Participants were admitted to the research ward on the night before the food challenge. They were allowed to sleep for a maximum of 2 hours during the night. All participants were woken by 3am regardless of whether they have slept and were kept awake by nursing staff who kept a log of the participant's activities every 15 minutes until the morning peanut challenge. Dosing was conducted in the same manner as the baseline challenge. Tiredness was assessed objectively

using the Psychomotor Vigilance Task²⁰⁰ and subjectively using the Karolinska Sleepiness Scale.²⁰¹ (Appendix 2)

No intervention challenge

This was conducted under the same conditions as the baseline challenge.

Ethics committee approval

This study was approved by the NRES committee East of England. Informed, written consent was obtained from all participants prior to participation in the study. An Independent Data Monitoring Committee consisting of a team of experienced allergists oversaw safety data and assessed severe reactions. RD approval at each site and CRF permissions were obtained.

Study oversight

There was a Trial Management Group (TMG) comprising principal investigators from each site, clinical fellows, nursing staff and a trial statistician who met on a fortnightly basis during study set up and then monthly thereafter to review project progress and safety.

An Independent Data Monitoring Committee (IDMC) consisting of clinicians and statisticians not directly involved in the study reviewed safety data and severe events reported by the Principal Investigators.

The Trial Steering Committee (TSC) consisted of an independent chair, representatives from patient organisations, a clinician with experience of undertaking oral food challenges and the Food Standards Agency. The TSC had overall responsibility for the scientific strategy and direction of the study as well as ensuring the study achieved its aims in a timely manner.

Funding

This study was funded by the Food Standards Agency, UK Government.

CHAPTER 3

PILOT STUDY AND ALTERATION TO INITIAL STUDY DESIGN

The pilot process which led to the finalisation of the above study protocols is detailed below.

Pilot Baseline Challenges

Double blind placebo-controlled food challenge (DBPCFC) is the gold standard investigation for diagnosing food allergy and for establishing the allergen threshold of reactivity. Assessing symptoms elicited during challenge and deciding whether or not they are objective is a critical part of a threshold finding study and variability in interpretation can adversely affect threshold estimation. Adopting a standard way of assessing symptoms including how to classify subjective or objective symptoms, allows comparison of outcome during DBPCFC. Moreover the scoring and stopping criteria designed for conducting food challenges can affect the threshold estimate of a study. Therefore the parameters for stopping and declaring a challenge should be prespecified in challenge protocols. The only published practice parameter on oral food challenge in existence at the commencement of the study was the PRACTALL consensus report for food challenge which had received broad consensus from US and European allergists hence the decision to follow these criteria.⁸²

Aims of the pilot baseline challenge study

- To determine whether the PRACTALL challenge stopping criteria which had been designed for use in other studies could be safely applied to the TRACE study

Methods

4 peanut-allergic participants underwent a pilot baseline challenge to peanut (active arm only) using the method described above (Main Study Method: Baseline Challenge). Challenges were scored using the PRACTALL consensus criteria. This scoring system indicates

symptoms and signs that may merit caution and aims to inform the investigator whether a dose should be delayed, repeated or that the challenge should be stopped. Symptoms and signs are scored using a traffic light warning system.

Results

4 peanut allergic participants, 3 female and 1 male, age range 18-41 underwent challenges. 3 participants developed objective symptoms based on the challenge stopping criteria which allowed their reaction threshold to be defined (Table 4). One participant developed subjective symptoms only but we took the decision to stop the challenge as this was the first one we had conducted. It was decided for safety reasons that further refinement of the criteria was needed. We felt that many of the original criteria were based on symptoms experienced by children during oral food challenge and required alteration to make them applicable to adults. Furthermore, some participants, during the pilot baseline challenges were experiencing a rapid evolution of respiratory symptoms. By adding further refinement to airway symptoms we felt that we could detect warning signs, prevent rapid progression to severe symptoms and thus enhance safety. In addition we incorporated the peak expiratory flow rate as a functional measurement. Gastrointestinal symptoms were also further defined in terms of their persistence. Based on the pilot challenge reactions the weighting of various symptoms was changed. In the original PRACTALL consensus criteria it was suggested that challenges could be stopped on the basis of green (mild) symptoms present for greater than 120 minutes. We regarded these as subjective symptoms and stopping a challenge for subjective symptoms increased the risk of a false positive test. Therefore we decided to base a threshold estimate on objective symptoms (yellow or red symptoms as previously described) only.

Outcome

The PRACTALL consensus criteria have been modified for use in this study

Pilot Exercise Challenges

Background

Anaphylaxis during exercise has been reported to occur during bouts of physical activity of varying intensities. This ranges from high intensity activity such as running or jogging to even ordinary physical activity such as gardening.¹³⁷ In a study by Pals et al it was shown that small intestinal permeability, assessed by sugar excretion, was increased after exercising at 80% VO₂ max for 60 minutes and not at lower intensities (40 and 60% maximal oxygen uptake).¹³⁶ However it would be impractical to exercise our participants at this intensity for the same duration on repeated occasions during a single challenge day.

Aims of pilot exercise study

- To determine an acceptable and tolerable intensity and duration of exercise for participants during the study
- Aim to imitate real life exercise
- Detect any ingestion of food ingestion on exercise capacity (healthy volunteers will undergo the exercise protocol with the placebo dessert matrix).

Pilot exercise method

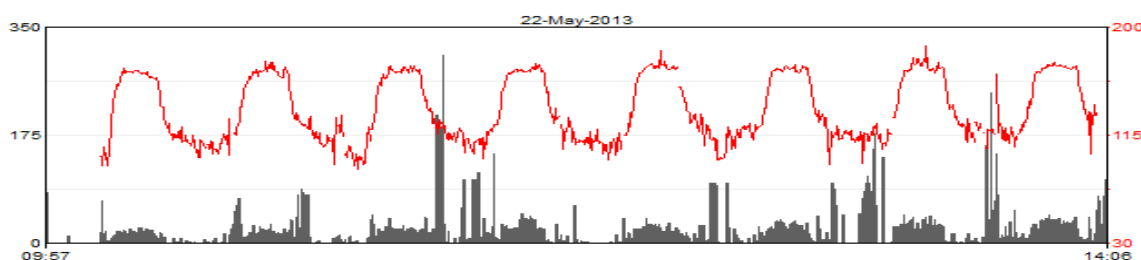
8 healthy volunteers of varying levels of fitness undertook a V02 max test to determine their maximum exercise capacity as measured by maximum oxygen uptake. ECGs were performed on all volunteers prior to exercise. Participants performed varying durations of exercise at 85% VO₂ max to determine tolerability. Exercise was performed initially on a treadmill but then latterly on a static bike for reasons outlined in results. In 5/7 healthy volunteers, placebo dessert matrix was given 5 minutes prior to each bout of exercise. Following finalisation of the protocol, 3 peanut allergic participants underwent a pilot exercise challenge to determine

safety. Blood lactate was measured during exercise, an elevated lactate is indicative of a normal physiological response to exertion. Dramatic increases in lactate characterise a normal response to exercise if a patient exceeds the work rate at which lactate can be removed from the blood as quickly as it enters the blood. Serum lactate was measured before and after each exercise bout.

Pilot exercise results

2 healthy volunteers undertook 8 x 15 minute bouts of exercise at 85% VO₂ max on a treadmill but this protocol was deemed to be too intense and unacceptable. 1 healthy volunteer undertook 8 x 5 minute bouts of exercise at 85% VO₂ max (treadmill) but this was deemed to be too easy and subjectively did not tire the participant. 4 healthy volunteers undertook 8 x 10 minute bouts of exercise at 85% VO₂ max (treadmill). One participant had to withdraw during the exercise due to a pre-existing knee injury. Otherwise this protocol was well tolerated and caused sufficient cardiovascular exertion. Ingestion of the challenge matrix did not affect exercise capacity. Due to logistical issues in obtaining a treadmill in the other study centre the exercise mode was switched to a static bike. The protocol of 8 x 10 minute bouts of 85% VO₂ max was piloted on 3 further healthy participants on a static bike. The exercise protocol was replicated with good results and a high heart rate was achieved during the bouts (Fig 2). Serum lactate was measured before and after each exercise bout and demonstrated an increase post exercise compared to pre exercise levels (data not shown).

Figure 2: Trace showing a participant's heart rate (vertical axis) during exercise bouts



Following an initial open baseline challenge with active peanut doses, 3 peanut allergic pilot volunteers undertook exercise challenges also with active doses. 2 performed the exercise challenge on a treadmill (prior to the change) and one performed the challenge on a static bike. 2 participants developed objective symptoms and one participant completed all 8 doses without reaction. For the 2 reactive participants there appeared to be an increase in reaction severity compared to their baseline challenge (Table 4). Both required treatment with adrenaline for severe symptoms which was not needed in their baseline challenges.

Table 4: Pilot baseline (n=4) challenges and pilot exercise (n=3) challenges.

Participant Number	Baseline Threshold Dose	Baseline Challenge Reaction Symptoms	Treatment Given	Exercise Threshold Dose	Exercise Challenge Reaction Symptoms	Treatment Administered
0115	5 (30mg)	OP, ECP, Na, AP, E (1)	IVAH, IVHy	5	OP, ECP, AP, Rh, Na, ERep (10)	IVAH, IVHy, Adr, IV fluids
0110	6 (100mg)	OP, ECP AP, Rh, U	OAH, OPr	6	OP, Na, PruG, Rh, EryG, Co, Wh	IVAH, IVHy, Adr, Salb
0109	6 (100mg)	OP, Ch,	OAH	Completed	OP	None
0108	5 (30mg)	OP, Rh, Na, E (1), EryL	IVAH, IVHy			

OP Oropharyngeal pruritus/tingling, PruG Generalised pruritus, ECP Ear canal pruritus, Ch subjective chest tightness Na Nausea persistent, AP Abdominal pain persistent, Rh Rhinorrhoea (persistent), U Urticaria localised, EryL Erythema Localised, ThT throat tightness, E Emesis, ERep Repeated emesis EryG Generalised erythema, Co Dry cough persistent, Wh Wheeze audible AI C altered consciousness OAH Oral antihistamines OP Oral prednisolone IVAH IV antihistamines IVHy IV hydrocortisone Adr Adrenaline Salb salbutamol

NB: Participant 0108 completed baseline challenge only and did not undergo exercise challenge.

Outcome

- It was decided that the optimal regime was for participants to undertake 8 x 10 minute bouts of exercise at an intensity of 85% VO₂ max during the exercise challenge days.

Pilot sleep deprivation challenges

Aims of pilot sleep deprivation study

- To determine an acceptable and tolerable amount of sleep restriction for participants.
- To aim to imitate sleep restriction in the community.
- To pilot the use of objective and subjective measures of tiredness namely the Psychomotor Vigilance Task and the Karolinska Sleepiness Scale.

Pilot sleep deprivation method

3 peanut allergic participants underwent a peanut challenge with active doses following restricted sleep of 3 hours.

Pilot sleep deprivation results

3 peanut allergic participants, 1 male and 2 female, age range 18-28 completed the pilot sleep deprivation challenges. All participants developed objective symptoms during challenge and reaction thresholds could be established (Table 5).

Table 5: Pilot sleep deprivation challenges

Participant Number	Sleep Dep Threshold Dose	Sleep Deprivation Challenge Reaction Symptoms	Treatment Given
0115	5 (30mg)	ECP, OP, Na, AP, E (1)	IVAH, IVHy
0121	5 (30mg)	OP, Co, Al C	IV AH, IV Hy, Salb, Adr
0126	6 (100mg)	OP, ThT, EryL, U	IV AH, OPr

OP Oropharyngeal pruritus/tingling, PruG Generalised pruritus, ECP Ear canal pruritus, Ch subjective chest tightness Na Nausea persistent, AP Abdominal pain persistent, Rh Rhinorrhoea (persistent), U Urticaria localised, EryL Erythema Localised, ThT throat tightness, E Emesis, ERep Repeated emesis EryG Generalised erythema, Co Dry cough persistent, Wh Wheeze audible Al C altered consciousness

OAH Oral antihistamines OP Oral prednisolone IVAH IV antihistamines IVHy IV hydrocortisone Adr Adrenaline Salb salbutamol

However 2/3 peanut allergic participants subjectively reported that they did not feel tired following the sleep restriction of 3 hours. 1 participant did subjectively report tiredness. Based on these pilot findings it was decided to further restrict the amount of sleep in the protocol to ensure adequate fatigue and to use formal objective and subjective measurements of tiredness (the Karolinska Sleepiness Scale (Appendix) and Psychomotor Vigilance Task). Therefore 3 further healthy volunteers were asked to pilot the protocol with 2 hours of sleep restriction. This amount achieved adequate levels of tiredness (Table 6) and was tolerated well.

Table 6: Psychomotor vigilance task results for 3 pilot healthy volunteer sleep

participants. A reaction time of greater than 300 milliseconds is classed as an impaired response time.

	Average reaction time (milliseconds)	Average false starts	Average missed signals
PRE sleep deprivation	278	1	1
POST sleep deprivation	357	0	2

Outcome

- Sleep restriction to 2 hours was decided upon for the final protocol.

Alteration to main study design: Placebo challenges

The intention of the original study design was to have a placebo arm for every active arm. The rationale of including placebos was to eliminate investigator or participant influence on the challenge outcome at every stage and also to validate the symptoms and signs which occur on active days.

However there were delays in starting the baseline challenges due to appointment of staff, obtaining local site permissions and additional piloting work, described above, for protocol refinement. It became clear that keeping to the original study design would result in challenge burden exceeding site capacity and the study not being completed by the funder deadline. Therefore as a study team we had to propose a study redesign to reduce the challenge burden

whilst maintaining scientific validity and participant safety. Various proposals were explored including eliminating an intervention (either sleep or exercise), removing placebos from some challenges, interspersing placebo doses within the active doses, removing ‘predictable’ placebos i.e. ones occurring on day 2 of the challenge pair or removing placebos from interventional challenges. We performed a sensitivity analysis looking at data from 29 baseline challenge pairs (placebo and active day) and 9 interventional challenge pairs (placebo and active) and found that there was no difference in threshold with the challenge method applied. The prediction of the researcher as to whether each day was placebo or active, based on the symptoms encountered was correct in every case. We also examined the symptoms and signs that occurred on placebo days. In every case these were minor and would not have triggered the challenge to be stopped or scored positive. Furthermore we observed that participants who received the active challenge on Day 1 of the challenge pair were essentially unblinded and were expecting to have an allergic reaction on Day 2 reducing the validity of the placebo arm.

It was decided that a placebo must be retained for the initial baseline challenge as this is essential for correct diagnosis in peanut allergy. However the decision was taken to remove placebos from the intervention arms. This also meant that each participant only had to attend 5 instead of 8 challenge appointments which also helped to improve booking challenges, study retention and reducing drop outs.

With no placebos, the participants would attend each interventional challenge knowing they would receive peanut. It could be argued that this would change their expectation and somehow alter their reporting of symptoms, compared to the original design. This might be true if subjective symptoms were being used to determine challenge outcome (e.g. itching or discomfort), however the stopping criteria used on active days required three concurrent ‘yellow’ symptoms or signs to be present. It is unrealistic to believe that such objective symptoms could be induced by a placebo effect, and our analysis indicated it is very unlikely to occur. A further criticism might be that comparing the original DBPCFC with later non-placebo interventional challenges would not be comparing ‘like with like’. However, this is controlled for in the study design as the main outcome is the difference between (baseline-repeat baseline) and (baseline-intervention). Both the repeat baseline and intervention challenges in this scenario will be internally controlled (neither have placebo).

CHAPTER 4

How do reaction thresholds vary as a result of sleep deprivation and exercise?

Introduction

Currently there are efforts by scientists, clinicians and food policy makers to model and manage allergen thresholds to help the food industry, and ultimately allow patients to make better informed and safe food choices. There is a lack of standardised international guidance on managing levels of unintentional contamination of food with allergens. Partly, this is due to a lack of knowledge about the exact level of allergen contamination which poses a problem to the food-allergic population. Several clinical studies have examined thresholds of reaction to allergen in food-allergic participants. From these, attempts have been made to model a population threshold. Peanut, probably due to its ubiquity and also its propensity to cause severe and fatal allergic reactions, has been the most widely studied. Taylor et al showed that peanut allergen elicitation thresholds can range from 0.5mg to 10000mg of whole peanut between peanut-allergic individuals.⁹⁸ There is clearly a wide intrinsic variation in patients' thresholds. However a possible factor influencing this wide apparent variation is that the contributory studies used to derive these estimates have varied in dosing regime, dosing interval, study entry criteria and food matrix.

Although it is widely known that thresholds vary across individuals in a population, little data exists on the variation of thresholds within allergic individuals over time. One study by Moneret-Vautrin suggests up to a 10-fold change in threshold with successive challenges (personal communication: Professor DA Moneret Vautrin and the North American and European branches of International Life Sciences Institute), while another suggests a small negative change in threshold of 0.81 fold in a control group of participants in an immunotherapy study who undertook a peanut challenge pre and post intervention (personal communication Dr Andrew Clark, STOP 2 study). It has been suggested that intra-individual variation may occur as a result of both host factors (associated atopic or comorbid conditions, age, medications) and co-factors (exercise, sleep deprivation, alcohol, infection and non-steroidal anti-inflammatory drugs). Indeed extrinsic factors, also referred to as cofactors, have been identified in 30% of anaphylactic reactions in adults.²⁰² Furthermore the influence of co-factors in causing reactions during immunotherapy to previously tolerated doses of allergen

has been well described. In a single study of peanut immunotherapy, six out of 20 individuals suffered from a clinical loss of tolerance if they took their maintenance doses when sleep deprived (for example <6 hours sleep overnight).¹⁴³ It is important that variation accounted for by co-factors is taken into account when undertaking statistical modelling of the population threshold. For this study two co-factors exercise and sleep deprivation were chosen as these were two, easily reproducible co-factors. Exercise has already been shown to have an effect in the clinically distinct condition Food Dependent Exercise Induced Anaphylaxis as discussed earlier and even light exercise such as walking or housework activities have been shown to be associated with allergic reactions. Sleep deprivation was chosen as there are no prospective data on how this modulates an allergic response and studies suggest that usual sleep duration has decreased substantially over time with an estimated 25-30% of the United States population sleeping 6 hours or less per night, an amount associated with significant health problems.²⁰³ Other factors were considered such as infection, alcohol and nonsteroidal anti-inflammatory drugs. Infection was rejected on the basis that although it would be apparent symptomatically it would be difficult to quickly confirm this via microbiological and virological investigation. There were also concerns that infection may be a very high risk co-factor given the concomitant effect of exacerbating asthma in asthmatics. With regard to alcohol it would be difficult to determine the amount to be administered to achieve the same physiological effect across individuals in respect to different tolerance levels amongst participants. Furthermore there was a concern that participants may be less compliant with the study protocol and may be less able to articulate their symptoms in an inebriated state hence raising a safety concern. Further the TRACE study is part of iFAAM, a food allergy consortium and other research groups are looking at other factors such as the effect of proton pump inhibitor use and the effect of the allergen matrix and allergen processing on allergic reactions. The aim is to eventually combine the data from these studies hence the rationale for not choosing these other factors to examine.

Chapter Hypothesis

Co-factors impact peanut allergy reactions by lowering reactivity thresholds.

Chapter aims

1. To establish a dose-distribution curve for peanut threshold in a UK peanut allergic population of adults.

2. Model the variability of challenge thresholds and severity over time within individuals as a result of repeat challenges.
3. Examine how co-factors (exercise and stress through sleep deprivation) shift the dose response curve and alter severity

METHOD

The full method has been described in detail in Chapter 2. The following is an abbreviated method.

Recruitment

Between 2013-2016 participants were recruited to the study hosted in the NIHR/Wellcome Trust Clinical Research Facility at Addenbrooke's Hospital, Cambridge. Participants were recruited through advertisements in local media and also from general allergy clinic.

Screening

An initial screening visit was undertaken to elicit a history of peanut allergy (a previous systemic reaction following ingestion of peanut) and evidence of sensitisation to peanut through skin prick tests and serum specific IgE to whole peanut and its components Ara h1, 2, 3, 8 and 9 (ImmunoCap Thermofisher). The presence of other comorbidities including atopic diseases and their control was assessed. In preparation for the exercise challenge, a VO₂ max test was performed to determine the participant's maximum exercise capacity. Participants were provided with sufficient time to consider written and verbal information related to the study. Written informed consent was obtained from participants before any study procedures were undertaken. Full study inclusion and exclusion criteria are shown in detail in Chapter 2.

Baseline challenge

Eligible participants were invited back for an initial baseline double-blind placebo-controlled food challenge (DBPCFC). On the day of the challenge a preliminary examination to assess fitness for the challenge was performed including spirometry, POEM score, asthma control test and vital signs. As per the PRACTALL consensus report, the challenge was only allowed to proceed if participants were considered to be stable regarding other atopic conditions. Treatment with antihistamines was withheld for 3-5 days prior to challenge. For safety, an intravenous line was inserted prior to challenge in order that treatment could be rapidly administered if required.

The DBPCFCs were undertaken as previously described, ingesting increasing doses of food matrix either alone or containing allergen until objective symptoms of an allergic reaction were seen. The dosing regimen is shown in Table 7. The starting dose was 3 micrograms of

peanut protein corresponding to 12 micrograms of whole peanut. Peanut allergy was confirmed by a positive outcome (objective symptoms) during DBPCFC. The dose at which the participant developed objective symptoms was recorded as their threshold. Participants with confirmed peanut allergy were then randomised to receive 3 further active peanut challenges (sleep, exercise and no intervention) in a random order.

Table 7: Study dosing regimen

Dose Number	1	2	3	4	5	6	7	8
Amount of peanut protein	3µg	30µg	300µg	3mg	30mg	100mg	300mg	1g

Exercise challenge

Participants were administered each dose 5 minutes after completing a 10 minute bout of exercise at 85% VO₂ max.

Sleep challenge

Participants underwent a night of restricted sleep (2 hours) and received the peanut challenge the following morning.

No intervention challenge

This was carried out in the same manner as the initial baseline challenge but with the active arm only.

Statistical analysis

The primary endpoint was the cumulative peanut threshold dose in mg of protein after each DBPCFC. Experimentally induced individual thresholds lie between two boundaries: the no observed adverse effect level (NOAEL) the highest dose observed not to produce any adverse effect and the lowest observed adverse effect level (LOAEL) which is the lowest dose that is observed to produce an adverse effect. Operationally the LOAEL is also referred to as the minimum eliciting dose or MED.²⁰⁴ In this study the LOAEL was the lowest cumulative dose of peanut protein which caused a reaction.

The primary endpoint of difference in the LOAEL was defined as the natural log of LOAEL at challenge minus the natural log of LOAEL at baseline.

$$= \text{Challenge log LOAEL} - \text{baseline log LOAEL}$$

The primary outcome was summarised by challenge type and challenge timing (median, IQR). The difference in threshold (logged) LOAEL between the non-intervention challenge and each intervention challenge (exercise and sleep deprivation) was estimated using a linear mixed effects model. Fixed effects included the challenge type (exercise, sleep deprivation, with non-intervention as reference), age, sex, timing of challenge, baseline threshold (logged), presence of asthma, centre and baseline Arah2. The primary analysis estimated the effect of type of challenge, against non-intervention, from the model along with confidence interval and p-value for whether the difference in log-LOAEL was significant.

Sample size estimation

As there were no published data on intra-individual variation in thresholds over time from repeat challenges different scenarios were considered with power assessed by simulation. In addition an example from the development of hypoallergenic infant formula in 1999 was used.²⁰⁵ It was suggested that low dose challenges should be conducted using a standardised protocol on a total of 29 subjects with a specific food allergy and if a level could be identified which did not elicit a reaction in any of the 29 subjects then statistically it could be inferred that there is 95% confidence that less than 10% of the allergic population would react to this amount of allergenic protein or less.²⁰⁶ Food challenges based on these numbers have become standard. However the sample size should also be sufficient to fit threshold distributions based on both the NOAEL and LOAEL. Klein Entink et al conducted a simulation study to evaluate the effects of sample size and dosing regimens on the accuracy of the threshold distribution curve (described below)²⁰⁷ and showed that the bias and accuracy of estimation improved the most with each step in sample size from n=20 to n=60. Therefore for the establishing a population threshold curve, a sample size of 60 was chosen.

Population threshold curve

A secondary objective of the study was to derive a population curve for the different challenge types (no intervention, exercise and sleep deprivation).

Performing challenges in all food allergic individuals in the general population is impossible, thus statistical models have been developed to construct a dose distribution curve, as

described by Crevel et al,²⁰⁸ together with parametric interval censored survival analysis used by Taylor et al for a specific allergen for individuals in a sample population.⁹⁸ From these curves it is possible to derive an ‘eliciting dose’ and estimate the dose likely to elicit reactions in a certain proportion of an allergic study population. For example, an ED10 is the dose which elicits a reaction in 10% of the allergic population.

Interval censored survival analysis (ICSA) is a statistical method which is used when there is uncertainty as to the exact time that units ‘failed’ within an interval. This methodology is appropriate when considering allergen threshold doses as the exact dose that provokes a reaction is not known but is known to fall into a particular interval i.e. between two doses.

Interval censored survival analysis permits the use of data points from first dose reactors (classed as left censored) and those failing to respond to the uppermost dose (right censored). If the participant had subjective symptoms only to the uppermost dose then the last dose was set as the NOAEL and the LOAEL was set to infinity. ICSA was carried out using the Survreg function in the statistical package, R.²⁰⁹

Data were analysed as cumulative doses and modelled using 3 distributions log-normal, log-logistic or Weibull as no biological or mathematical reason exists to favour one over the other. This model was used to find the dose predicted to provoke reactions in different proportions of the peanut allergic population (ED1, ED5, ED10, ED50, ED80, and ED95). However the reference dose is not a simple mean of the chosen EDs from each distribution but requires expert judgement. When selecting the recommended reference dose, weight is given to the best statistical fit for each parametric model (as determined by log likelihood), as well as visual examination of the fitted probability dose distribution curves to the actual individual threshold data within the low dose zone which are the most important doses with regards to public health protection.

Once ED estimates were calculated, confidence intervals for these levels were calculated by taking the 2.5th and 97.5th percentiles of the bootstrap distribution of the EDs.

Dose distribution curves together with eliciting doses were modelled for each challenge type: baseline, sleep deprivation, exercise and no intervention.

RESULTS

Study Population

Study recruitment took place between May 2013 and September 2016. There were 232 potentially eligible website registrants and 35 potentially suitable recruits from the Allergy clinic at Addenbrooke's Hospital. Ninety seven participants were screened. Out of the 97 screened participants, 75 (77%) were eligible to attend for baseline challenge. Sixty participants undertook baseline challenges. Positive DBPCFC confirmed the diagnosis of peanut allergy in 57/60 subjects (95%), 3/60 participants reacted with only subjective symptoms during the entire challenge. Three further participants had to be excluded following baseline challenge, 2 participants suffered severe reactions at baseline and were therefore excluded on safety grounds and 1 participant because she disliked the taste of the dessert matrix. Fifty four participants were randomised to attend for the intervention challenges. In total 41 participants completed the full study (all 5 challenges) and are referred to as the per-protocol study population. Several more participants attended for at least 1 intervention and this group is referred to as the full analysis population (No intervention challenge, n=44, Exercise n=43, Sleep Deprivation n=43). To maximise the data available the analyses in this chapter are based on the full analysis population. The per-protocol results are included in Appendix 3 and are reflective of the full analysis population results.

For the second part of the analysis which involves the production of the threshold distribution curves and the derivation of the population thresholds (ED estimates), the extended analysis population was used. This refers to all participants who underwent a baseline challenge and includes all the participants who were excluded at baseline and also right censored individuals. For comparison of the population threshold curves for the intervention challenges, the full analysis population was used. The characteristics of the extended analysis population are shown in Table 8.

Table 8: Baseline clinical characteristics of extended analysis population (n=60). Values are presented as medians where shown.

Clinical characteristic	Values
Age (y)	21
Female sex (%)	50
Asthma (%)	52
Rhinitis (%)	82
Eczema (%)	33
Age of onset of allergy (y)	3
Number of previous reactions	4
Adrenaline use during most severe historical reaction (%)	25
Wheeze during most severe historical reaction (%)	23
Peanut SPT wheal diameter (mm)	10
Peanut specific IgE (kU _A /L)	11.9
Arah2 specific IgE (kU _A /L)	9.14

Establishing NOAEL and LOAEL

Baseline challenges

For the extended analysis population, no objective symptoms occurred on the first dose of the challenges thus no participants were left censored. The lowest median cumulative LOAEL of peanut protein for objective symptoms was 133.333mg (IQR 33.333-433.333mg). The median cumulative NOAEL was 33.333mg. Three participants experienced only subjective symptoms to the last dose. These participants were therefore right censored. One of these participants proceeded to open challenge with whole peanut in a food challenge clinic and was reactive at a dose of 16 whole peanuts, equal to 2400mg peanut protein (higher than the upper limit of the study dose range (1433.333 mg)). A paired T test was used to compare the means of the baseline challenge (230.2mg) and No Intervention challenge (202.4mg) and

showed no statistical difference between the two means (p value 0.22). For the full analysis population the median cumulative LOAEL was 133.333mg (IQR 33.333-433.333mg).

Correlation of threshold dose with participant characteristics

There was a significant correlation of the age of onset of peanut allergy with threshold dose with an older age of onset being associated with a higher threshold dose. A significant correlation was also demonstrated with the severity grade of the most severe historical reaction and threshold dose. There was a significant inverse correlation of the markers for sensitisation (peanut specific IgE and Arah2 specific IgE) with the threshold dose at baseline challenge (Table 9) but peanut SPT wheal size could not be correlated. The number of adverse reactions to peanut and age at study entry could not be correlated to threshold dose at baseline challenge.

Table 9: Relationship between threshold dose and clinical characteristics (n=57, p<0.05)

Comparison	Correlation coefficient	Significance
Age at study entry	0.15	0.27
Age of onset of peanut allergy	0.37	0.007
Severity grade of most severe reaction to peanut	0.29	0.03
Number of adverse reactions to peanut	-0.12	0.39
Skin prick test to peanut (wheal in mm)	-0.09	0.52
Peanut specific IgE	-0.28	0.03
Arah2 specific IgE	-0.30	0.02

The effect of sleep deprivation and exercise on challenge threshold

The median cumulative threshold dose reached for the no intervention challenge was 133.333mg (IQR 33.3-433.333). In both exercise and sleep deprivation challenges the median cumulative threshold dose reached was lower: exercise 63.333mg (IQR 33.333-133.333mg) and sleep, 33.333mg (IQR 33.333-133.333) respectively compared to the dose reached during the no intervention challenge. Figure Plots are provided on the dose scale (Figure 3a) and log

dose scale (Figure 3b). Using a linear mixed effects model, a significant effect of exercise and sleep deprivation on the change in log-threshold from the no intervention challenge was observed with a more pronounced effect with sleep deprivation (Table 10). The estimated changes in log-threshold for exercise and sleep (95% confidence intervals) were -0.311 (-0.636,0.013; p=0.060) and -0.755 (-1.079,-0.43; p=0.000) respectively. Random effects for each participant were included in the model: challenge type (exercise, sleep-deprivation), age, sex, timing of the challenge, baseline log-threshold, presence of asthma and baseline Arah2. The only significant effect was of visit number.

Table 10: Estimated effect, 95% confidence interval and p-value for each term in the linear mixed effects model. Full analysis population.

Variables	Estimate	CI	pvalue
Baseline LOAEL	-0.001	(-0.001,0)	0.136
Non- intervention	NA	(NA,NA)	NA
Exercise	-0.274	(-0.59,0.041)	0.088
Sleep	-0.703	(-1.024,-0.383)	0.000
Visit1	NA	(NA,NA)	NA
Visit2	-0.133	(-0.448,0.182)	0.403
Visit3	-0.448	(-0.768,-0.127)	0.007
No Asthma at baseline	NA	(NA,NA)	NA
Asthma at baseline	-0.341	(-0.878,0.196)	0.206
Arah2	0.005	(-0.005,0.014)	0.303
Female	NA	(NA,NA)	NA
Male	0.041	(-0.516,0.598)	0.882
Age	-0.016	(-0.055,0.023)	0.419

Figure 3(a): Dose reached (mg peanut protein) by challenge for full analysis population

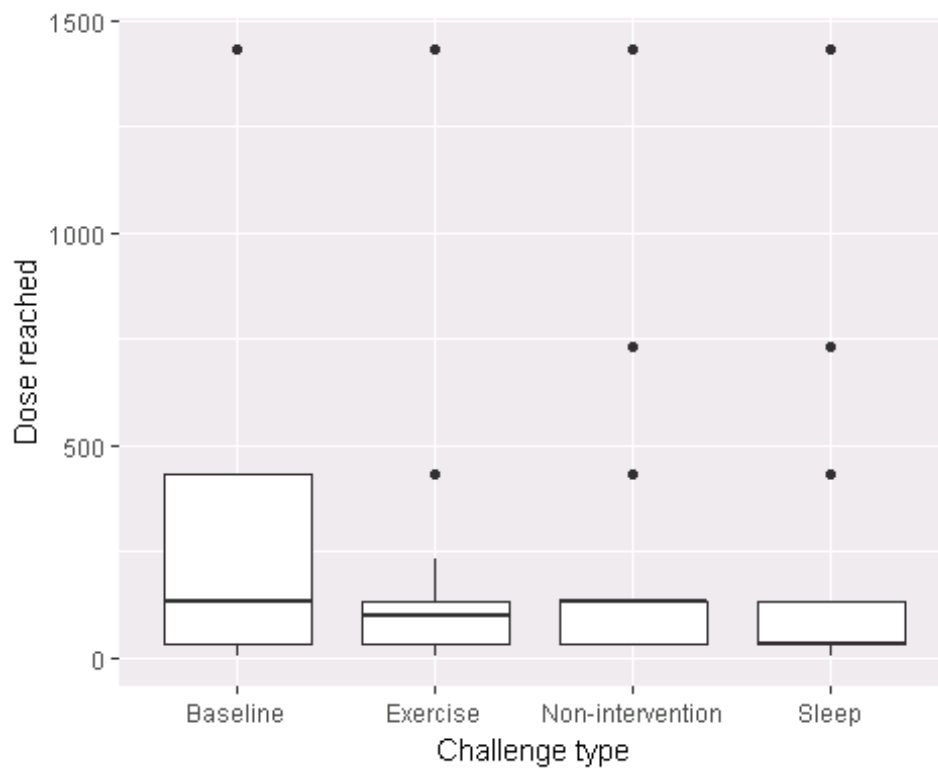
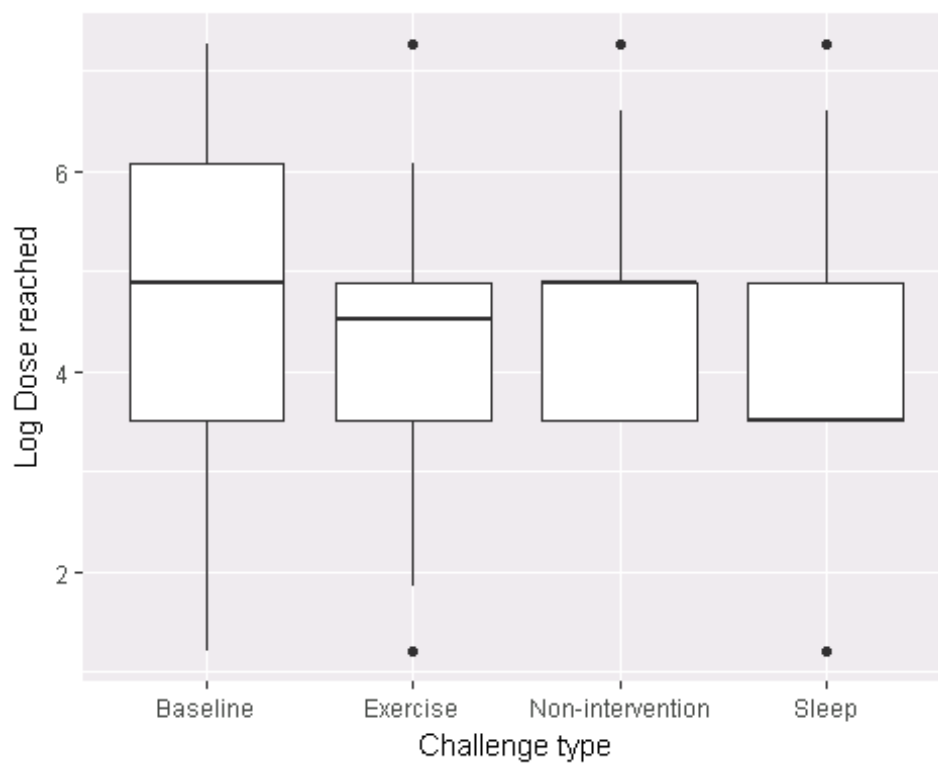


Figure 3(b): Log (dose reached) by challenge for full analysis population



The effect of visit number on challenge threshold

The cumulative threshold dose reached by visit number is shown in Figure 4a and 4b. There is a decreasing trend in the median threshold over time with a significant decrease in threshold at Visit 5 (final intervention challenge) compared to Visit 3 (first intervention challenge), Visit 5 median 33.333mg (IQR 33.333mg-133.333mg) compared to Visit 3 median 133mg (IQR 33.333mg-133.333mg).

Figure 4(a): Plot of dose reached (mg protein) by visit for full analysis population. The three intervention challenges (sleep, exercise and no intervention) are referred to as Visit 3,4 and 5.

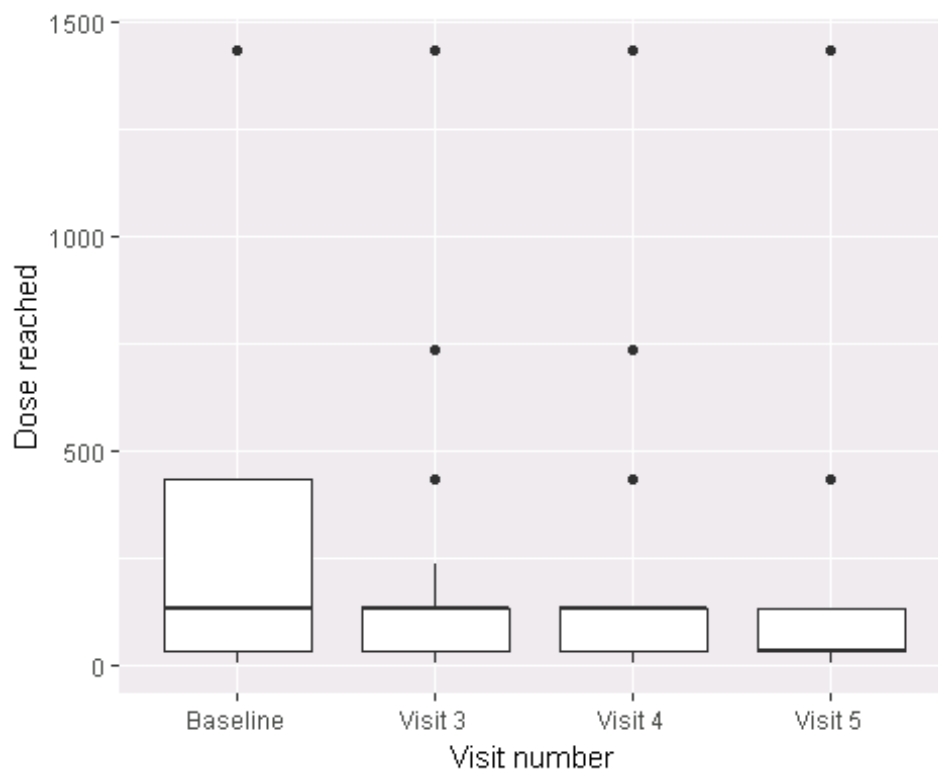
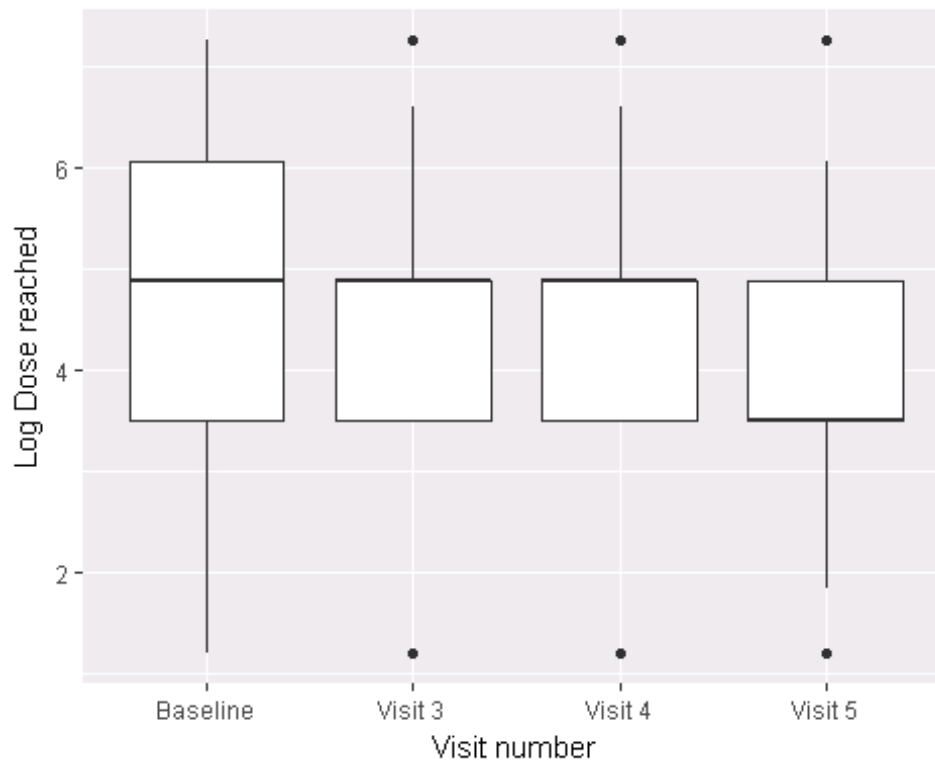


Figure 4(b): Log (dose reached) by visit for full analysis population. The three intervention challenges (sleep, exercise and no intervention) are referred to as Visit 3,4 and 5.



Population threshold curves

Log-normal, log-logistic and Weibull probabilistic distribution models were fitted to the data and no significant differences were found between the three models. Using the Akaike information criterion (AIC), a value was calculated for each model, a lower AIC suggesting a relatively better fit between candidate models. The log-normal model, which fitted the data best was used. Curves were derived for each challenge type and are shown. Figure 5 displays the threshold distribution curve for the extended analysis population which consisted of all individuals who underwent a baseline challenge and included non-randomised individuals. Threshold distribution curves for the Full Analysis Population are shown in Figure 6 and included all individuals who underwent at least 1 baseline challenge. Cumulative EDs were extrapolated from the models and are listed below. In the Full-Analysis set (Table 11), the ED10 and ED5 for baseline challenges estimated using the log-normal distribution model for all peanut allergic participants was 12.2mg peanut protein (95% CI 7,20.8) and 7.5mg peanut protein (CI 4.1, 13.2). The ED10 and ED5s in the extended analysis set (Table 12) which

includes the right censored individuals were similar, 12.3mg peanut protein (CI 7.3,20.4) and 7.6mg peanut protein (CI 4.3, 9.7) respectively.

The effect of co-factors (exercise and sleep deprivation) on eliciting doses

The eliciting doses are shown in Table 11. Using the ED10 as an example, compared to the No Intervention challenge (15.5mg 95% CI 10, 23.7) the ED10s for exercise (6.3 mg 95% CI 3.4,11) and sleep deprivation (3.2mg 95% CI 1.7,5.8) were lower. The ED10 for exercise was nearly 2.5 times lower and sleep deprivation 5 times lower.

Figure 5: Dose reaction curve for baseline challenge, extended full analysis set. The dashed lines indicate the 95% confidence interval.

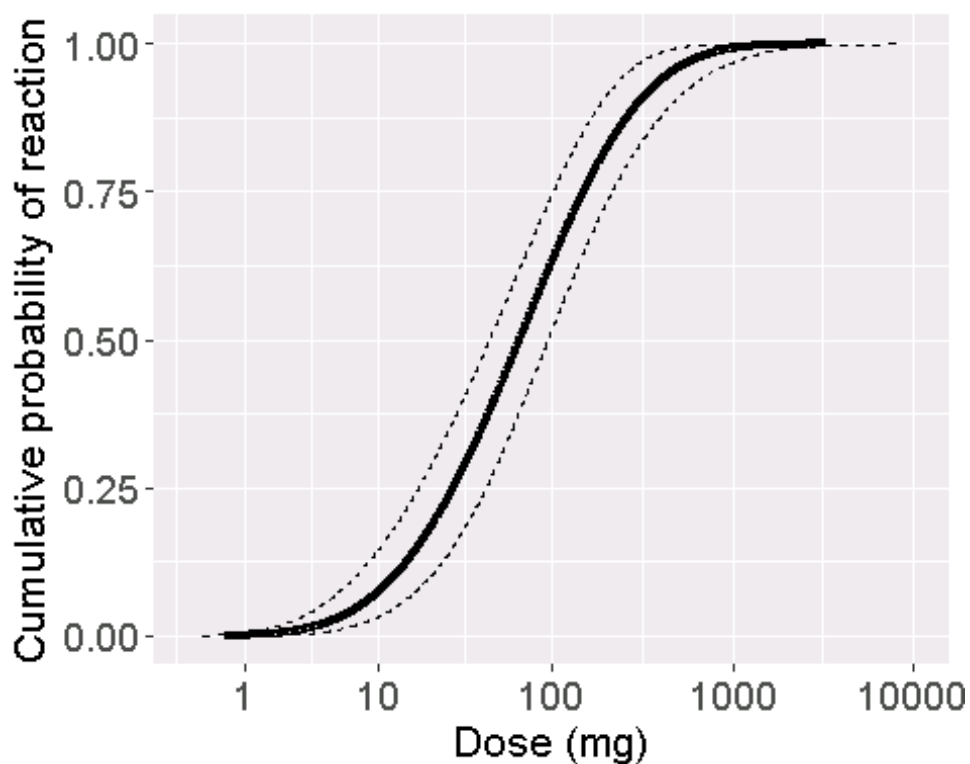


Figure 6(a): Dose reaction curve for non-intervention challenge, full analysis population.

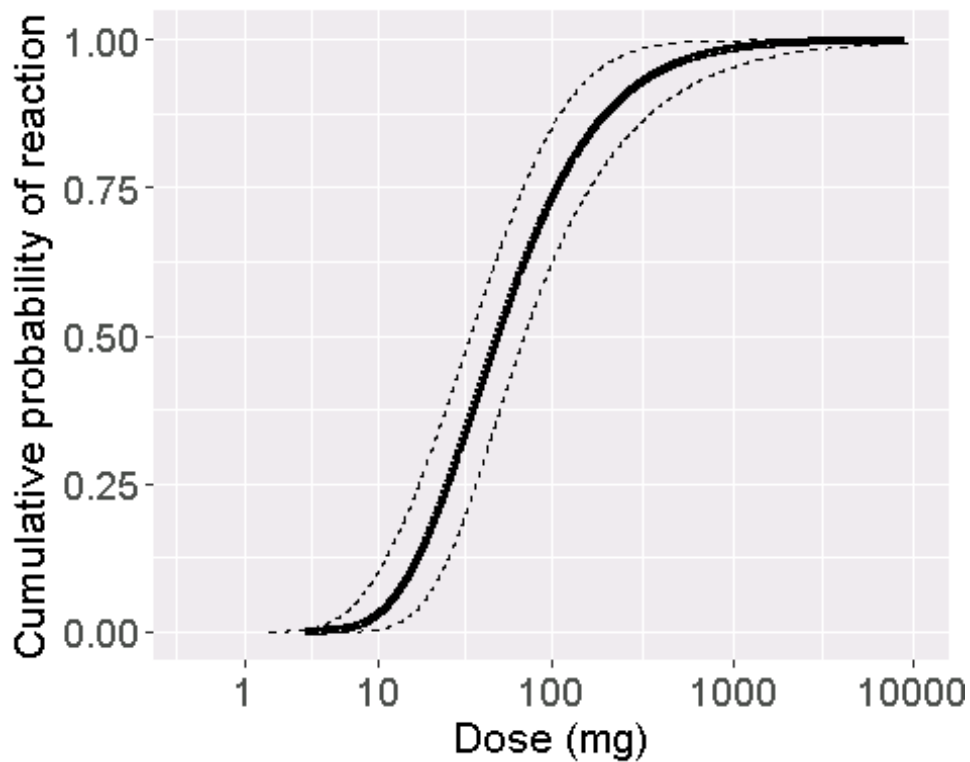


Figure 6(b): Dose reaction curve for sleep challenge for full analysis population.

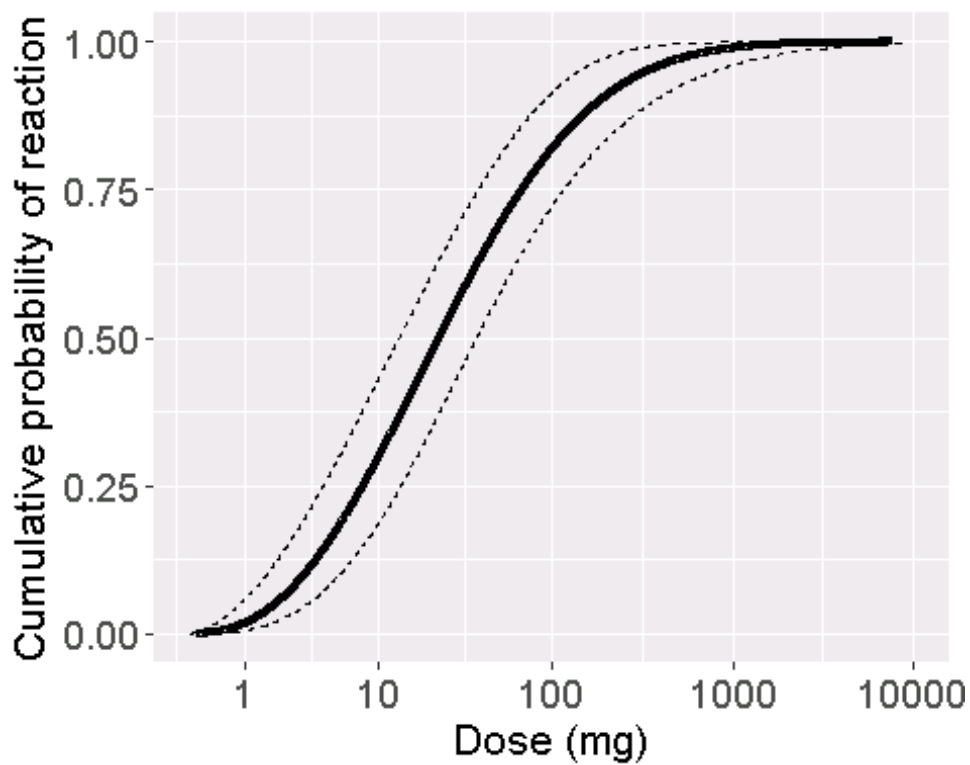


Figure 6(c): Dose reaction curve for exercise challenge for full analysis population.

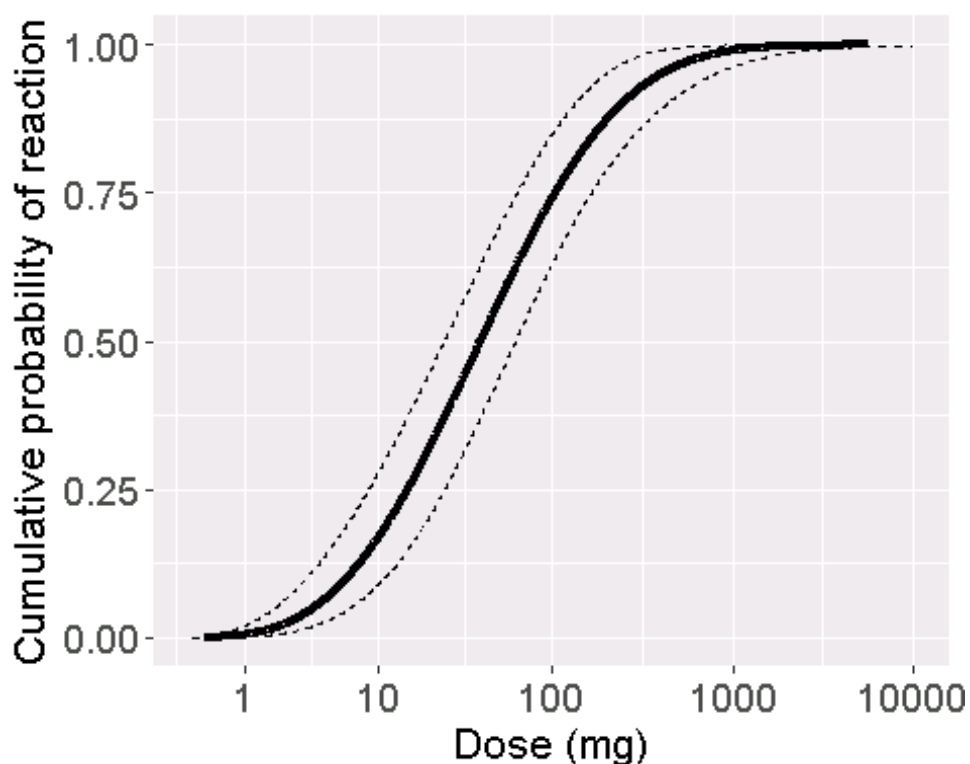


Table 11: Eliciting Doses (and 95% CI), full analysis population

	Baseline challenge	Non-interventional challenge	Sleep challenge	Exercise challenge
ED1	3 (1.5,5.6)	7.4 (4.4,12.3)	0.7 (0.3,1.4)	1.5 (0.7,2.7)
ED5	7.5 (4.1,13.2)	11.8 (7.3,18.6)	1.9 (0.9,3.5)	3.8 (2,6.8)
ED10	12.2 (7,20.8)	15.5 (10,23.7)	3.2 (1.7,5.8)	6.3 (3.4,11)
ED50	66.4 (44.3,99.3)	49.1 (34.5,69.6)	22.5 (13.4,37.5)	39.1 (24.5,62)
ED80	188.6 (123.8,286.9)	129.9 (81.1,207.8)	87.1 (48.6,155.4)	129.4 (78.6,212.6)
ED95	475.6 (267.3,845.4)	407.2 (180.8,915.3)	327.4 (138.7,771.4)	392.7 (196.4,784.3)

Table 12: Eliciting Doses, extended full-analysis set

	Baseline
Dose	challenge
ED1	3.1 (1.6,5.6)
ED5	7.6 (4.3,13)
ED10	12.3 (7.3,20.4)
ED50	64.8 (44.3,94.4)
ED80	178.9 (120.7,264.8)
ED95	438.7 (256.7,749.2)

DISCUSSION

This study provides the first systematically generated threshold data for a U.K peanut allergic adult population and is the first study to demonstrate that co-factors such as sleep deprivation and exercise lower reactivity thresholds. Furthermore, an inverse correlation between eliciting dose and sensitisation markers such as specific IgE to peanut and Arah2 is shown as well as a positive relationship between the number of previous accidental exposures and severity of previous reaction.

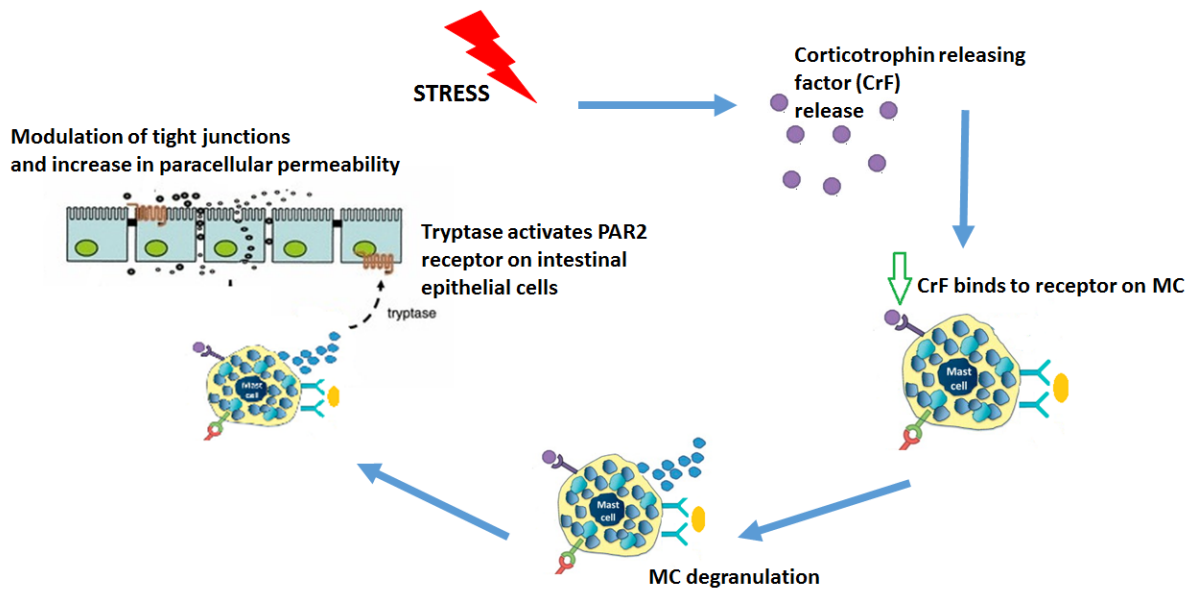
By using a dosing regimen with a low starting dose of 3 micrograms, a NOAEL was confidently established as there were no first (left censored) dose reactors. In addition, by using modified symptom severity grading criteria true thresholds based on objective symptoms were identified. Previous studies have often based threshold estimates on subjective symptoms which could lead to an underestimation of the true threshold dose.¹¹⁹ Three participants were able to tolerate the top dose (right censored) however this was a minority of subjects (5%). These three right censored individuals did not affect median cumulative threshold dose of peanut protein which was the same for the extended analysis population (including these individuals) as for the full analysis population (excluding these individuals). The median cumulative threshold of peanut protein in our sample was 133mg which is approximately equivalent to the amount of protein contained in 1 whole peanut seed (150-200mg).

In this study, a fall in the median cumulative threshold dose for peanut occurred when participants were subjected to exercise and sleep deprivation compared to receiving a peanut challenge with no intervention applied. These data support the hypothesis that co-factors augment anaphylaxis. Reports from the literature suggest that exercise as a cofactor plays a role in 0-15.9% of anaphylaxis cases.²¹⁰⁻²¹² However this estimate is based on retrospective analyses of implicating factors in anaphylactic reactions. Previously it has been shown in a clinically distinct condition, Food Dependent Exercise Induced Anaphylaxis that exercise can act as an augmentation factor in patients who can otherwise tolerate wheat containing products when given alone.²¹³ However the effect of exercise in patients who have established allergy and cannot tolerate the allergen under any circumstance has not previously been shown. It is possible that exercise exerts its effect through under-perfusion of the gut

resulting in a relative ischaemia with resultant damage to tight junction integrity. This may lead to increased permeability of the gut to food allergens.¹³⁷

Sleep deprivation resulted in a more pronounced lowering of the reactivity threshold compared to exercise. The effect of sleep deprivation, in this case used as a proxy for stress, has never prospectively been studied in allergic reactions. As mentioned previously, it has been noted in immunotherapy studies that a loss of tolerance to peanut can occur in the maintenance phase when subjects consume peanut doses whilst tired or stressed. The underlying mechanism may also be due to enhanced gastrointestinal permeability. It has been shown in animal models of inflammatory bowel disease that stress results in enhanced intestinal permeability.²¹⁴ Both acute and chronic stress have been shown to increase ion and water secretion and intestinal permeability in the jejunum and colon of laboratory animals.²¹⁵ These changes were associated with a significant increase in the permeability of the epithelium to macromolecules. It is well known that under stressful circumstances such as acute sleep loss, corticotrophin releasing factor (CrF) is released signalling the first step in the activation of the HPA. This hormone has potent effects on the gut via modulation of inflammation, increase in gut permeability and modulation of gut motility.²¹⁶ The translation of stress signals to gut mast cells may also play a pivotal role. Mast cells have surface receptors for CrF which may be an important indication of the link between stress and these cells. Mast cells in the GI tract serve as end effectors of the brain-gut-axis (BGA). When the brain gut axis is activated mast cells release a wide range of mediators including mast cell tryptase, histamine, heparin and PAF.²¹⁷ Tryptase can activate PAR2 receptors on epithelial cells resulting in modulation of tight junction proteins and increases in permeability through paracellular pathways in the intestinal epithelium.²¹⁸ PAR2 receptors have also been found on mast cells, thus activation of PAR2 can propagate the release of proinflammatory mediators from nerve endings, potentiating mast cell degranulation and creating a positive feedback loop.²¹⁹ Stress did not affect gut permeability in MC-deficient rats²¹⁶ supporting the critical role that mast cells play in orchestrating the stress response in the gut (Figure 7).

Figure 7: The effect of stress on mucosal mast cells and intestinal permeability



There is evidence of an effect on threshold with an increasing number of visits. A significant lowering of threshold in the final intervention visit compared to the first intervention visit was seen. Indeed in a study by Wainstein et al where follow up oral food challenges were performed in peanut allergic children who had been challenged at 35.5 (mean) months earlier, a decrease in threshold was noted in 10/13 patients, probably reflecting a high rate of natural resolution in their cohort of patients.²²⁰ However we did note that there was some reproducibility in threshold between the baseline challenge (median cumulative threshold dose 133mg peanut protein) compared to the no intervention challenge (median cumulative threshold dose 133mg peanut protein) even accounting for the fact that the baseline challenge was placebo controlled and the no intervention was not. Knulst et al have also reported that there is reasonable inter-challenge consistency when people have a second identical challenge 1-2 years after their first challenge.²²¹ However Glaumann et al found no threshold reproducibility in children who received 2 successive peanut challenges.¹²⁶ The relationship with reaction severity over time will be discussed in the next chapter. Although the lowering of threshold dose over time may be a true phenomenon it is possible that the study design may have exerted some effect. It is possible that the participant learns their reaction over time and anticipates the development of symptoms. Similarly the investigator may act in a more cautious way with recall of the participant's previous reaction. However the study was

designed to minimise this bias by ensuring that the participant was deemed to have reached their reaction threshold with only the appearance of clear objective symptoms.

The relationship between the degree of sensitisation and threshold has previously been studied. In our study, a higher peanut and Arah2 specific IgE level correlated with a lower elicitation threshold. A correlation between skin prick test wheal and threshold dose could not be demonstrated. This inverse correlation has been noted in paediatric studies¹⁰¹ and more recently in an adult population.²²² Other studies, however, have failed to show such a correlation perhaps due to differences in the recruited population.¹²¹ A relationship between the severity grade of the most severe historical reaction and eliciting dose has also been identified in our study i.e. participants who had suffered more severe reactions tended to have a higher eliciting dose at challenge. This is directly in contrast to Blumchen *et al* who showed that patients who had more severe symptoms during their worst accidental reaction had lower eliciting doses at challenge.¹⁰¹ The present study is the first to demonstrate a correlation between the age of onset of peanut allergy and eliciting dose, with an older age of onset being associated with a higher eliciting dose at challenge. Unlike other studies, an association between the number of previous accidental exposures and eliciting dose at challenge could not be found.¹⁰¹

Using the data from individual participant threshold doses we were able to estimate a population threshold using dose distribution modelling. Population eliciting thresholds can be used by governments and the food industry to help inform policy and precautionary labelling. We identified an ED10 of 12.3 mg of peanut protein and ED5 7.6 mg of peanut protein. When including the 3 right censored individuals in the analysis there was a minimal effect on the dose distribution curve and therefore threshold estimate. In comparison to other studies, our ED estimates were similar to Klemans *et al* who derived ED10 and ED5 threshold estimates 10.8mg and 5.08mg peanut protein respectively.¹²⁴ However our estimates were much higher compared to most other studies where the ED10 estimates range from 2.8-4.42mg.^{101,102,108,113,115} A possible reason for this difference could be that the majority of other studies are based on data from children. It has previously been reported that there are differences in the threshold distribution curves of children compared to adults with peanut allergy are different with children exhibiting a greater sensitivity than adults. However when the ED10 and ED5 estimates were compared for both groups little difference was found.¹²⁴ The most likely explanation for our much higher estimate, however, are stopping criteria and study population. In our study three definite concurrent objective symptoms were required to

stop the challenge and establish the threshold. Eller et al proposed an ED₁₀ of 32.9mg for their population of peanut allergic children however it is possible that this was due to a high starting dose of 85mg of peanut protein in their DBPCFC.¹¹⁶

Other studies simply use the appearance of a single objective symptom to establish a threshold^{101,102} or ‘any’ symptom including successive subjective symptoms.¹²⁴ In terms of study population, we included only those with a history of a systemic reaction who demonstrated sensitisation to major components (Arah2) whilst excluding those participants who were predominantly sensitised to Arah8 or monosensitised to Arah9. Some studies included participants who have never ingested peanut and are sensitised only.¹²⁴ Other studies have not stipulated that the history must be of a systemic reaction may therefore have included patients with oral allergy syndrome who display a milder phenotype.¹¹⁹ Thus it can be seen that directly comparing threshold studies is problematic.

This study is the first to establish population eliciting doses for peanut when participants are subjected to co-factors: sleep deprivation and exercise. Furthermore we are able to relate these to an index threshold estimate when no co-factor (No intervention) is applied to calculate the magnitude of the effect. This calculation can be utilised by regulatory authorities when attempting to calculate an adequate safety net for reference dosing. Currently in allergen risk assessment, regulatory authorities have been guided by regulatory toxicology practice determining safety or uncertainty factors. Once a threshold dose has been established, an uncertainty factor is applied to account for variability. In toxicology studies, an uncertainty factor of 10 is applied when extrapolating data from animals or observations on human subjects to determine acceptable daily intake. In addition to this, another factor of 10 is often applied to account for human to human variability giving an overall uncertainty factor of 100 providing a very conservative safety net. However by determining the magnitude of variability caused by co-factors, this study has enabled an uncertainty factor to be better tailored to allergic individuals hopefully establishing a more realistic reference dose. This study has also been instructive in terms of advice to patients. When patients are treated with peanut immunotherapy in our centre, they are advised to leave an interval of 2 hours between ingestion of their peanut doses and subsequent exercise. Furthermore, if the patients have suffered a period of sleep loss (for example due to jet lag or sleepovers) they are advised to omit the peanut dose. In general allergy clinic, we have also observed that cofactors may be implicated in reactions in patients who are allergic to lipid transfer protein. Allergy to lipid

transfer protein is widely reported in the Mediterranean population but less so in northern European countries such as the UK.⁶³

A limitation of this study is that our derived eliciting dose estimate is based on a controlled clinical study with a selected, well characterised peanut allergic population. Although patients were included with a history of anaphylaxis and historical adrenaline use, this study population may not be completely representative of the peanut allergic population at large and there may be selection bias in that patients who have suffered the most severe reactions in the community may be more reluctant to enrol in the study. However this is only a problem if one assumes that patients who suffered more severe reactions in the community represent the more sensitive (i.e. lower dose reactors) however our data demonstrate the opposite effect. Furthermore community exposures to peanut could be larger and more sudden than the gradual incrementally increasing allergen exposure in our protocol thus further data is needed on individual consumption patterns of high risk foods. Food matrix is known to have an effect on threshold dose, with higher fat matrices delaying absorption of allergen and ultimately resulting in higher cumulative doses of allergen.⁴⁰ However an ultimate aim is to combine these data with data from other studies using a variety of matrices which will average out the differences between the challenge vehicles. In this study partially defatted roasted peanut flour was used and previously authorities have questioned whether this differs significantly from whole peanut. Allen et al had sufficient data to allow a comparison of ED₅ values for challenges using pulverised peanut and others using partially defatted peanut flour and found no significant difference between the two sources.¹⁰⁸ Lastly our study population were predominantly students and a broader demographic would have been preferable. However in fatal anaphylaxis enquiries, deaths tended to be more common in this age group²²³ perhaps due to more risk taking behaviour²²⁴ thus it may be of benefit that the model is based on this age group.

In conclusion, in this study an ED estimate for a well characterised UK adult peanut allergic population was identified and for the first time it has been shown that co-factors such as sleep deprivation and exercise can lower an allergen reactivity threshold. This study has important public health impact through helping food policy makers and the food industry provide harmonised guidance on allergen labelling.

CHAPTER 5

The effect of repeat challenges on reaction severity and symptoms

Introduction

The severity of allergic reactions is influenced by several factors which are both host and event specific making allergic reactions unpredictable. This changeability is of concern given the propensity of peanut allergy to cause severe and fatal reactions.¹⁶⁵ This lack of stability in allergic reactions over time can add to anxiety for patients in managing their own allergy and uncertainty for clinicians in advising patients. A further practical implication is that oral food challenge, the current gold standard of food allergy diagnosis, carries with it inherent risk of invoking severe allergic reactions with one study reporting that 28% of these tests resulted in systemic and potentially life-threatening reactions.⁸⁴ There are limited data on the variability of reactions from one to the next within individuals particularly with regards to severity. Most information comes from patient recollection of symptoms occurring during reactions following accidental exposures which are anxiety provoking events and may be subject to recall bias. Even less information is available on the change in severity with repeat food challenges²²¹ as normally, food challenges are used to establish diagnosis and once an allergy is confirmed most patients do not undergo subsequent challenges. Therefore fine grained, prospectively collected detail on the evolution of symptoms during challenge has not been reported. Knowing this information would be useful to allergic individuals to help guide them about potential warning symptoms and the timely administration of treatment. Physicians will find the information helpful when advising patients about the potential severity of accidental reactions, and of ways to reduce risk.

Furthermore there is a lack of consensus about how to grade severity, with several different grading systems proposed. It has been reported that this is due to patients not presenting with a consistent constellation of allergic symptoms²²⁵ as well as debate about how much weight to attribute to certain symptoms, for example, comparing gastrointestinal to extensive cutaneous symptoms. Attempts have been made to determine clinical and laboratory predictors of severity on the basis that if higher risk allergic individuals could be identified then there could be enhanced surveillance and education of this group.

Chapter Hypotheses

Repeated challenges with peanut will result in a change in reaction severity with successive visits.

Symptoms patterns during allergic reactions are unique to individuals but with the application of co-factors there may be a change in symptom pattern, severity and frequency.

Chapter Aims:

1. To determine whether reaction severity changes over time within an individual as a result of repeated challenges
2. To assess whether co-factors influence reaction severity independently.
3. To examine whether symptom patterns are similar within individuals at each challenge and analyse whether there is commonality in symptom pattern across individuals in our population.
4. To determine whether there are any participant level characteristics such as asthma which predict severity of reaction.
5. To examine the perception of severity from a participant and investigator point of view and how these correspond with each other and also with the overall challenge severity score.

Methods

Challenge method

The challenge procedure including symptom stopping criteria has been described previously (Chapter 3).

Challenge treatment

In order to harmonise the management of positive reactions during challenge a treatment algorithm was used. This was delivered in accordance with symptom severity. The recommended treatment for red symptoms was with intramuscular adrenaline (0.5ml of 1:1000 (0.5mg)) administration. If there was a failure of improvement of symptoms, a second dose of adrenaline could be administered after 5-10 minutes. If there was still no resolution the critical care outreach team was alerted. With red respiratory symptoms, nebulised salbutamol (5mg) was co-administered. Nebulised adrenaline (1mg in 5ml 0.9% N. saline) was used as an adjunct treatment with very severe respiratory symptoms failing to respond to

initial bronchodilators and for significant laryngeal symptoms such as hoarseness, voice change or stridor. If there were severe generalised cutaneous symptoms suggesting extensive vasodilatation, persistent emesis or diarrhoea or hypotension intravenous fluids (Normal saline 0.9%) was administered.

For milder symptoms intravenous or oral antihistamines (chlorpheniramine 10mg or cetirizine 10mg respectively) with intravenous or oral corticosteroids (hydrocortisone 200mg or prednisolone 30mg respectively) were administered.

If participants suffered extremely severe reactions it was deemed unsafe for them to continue, they were excluded from the study.

If two or more doses of IM adrenaline were given to a single participant due to inadequate response to the first dose on 2 separate occasions, they were excluded.

To safeguard against late reactions participants were discharged with an adrenaline auto-injector, antihistamines, a short acting beta-agonist (asthmatics only) and a detailed plan of how to treat late symptoms.

All treatments administered and the frequency of adrenaline use for all challenges was reported.

Grading severity of reactions

There are various grading systems used to report systemic reactions but none are globally accepted. These proposed systems typically present 4 or 5 different grades of severity and are limited in discriminating reactions of varying severities especially towards the moderate and moderate-severe end of the spectrum. In order to overcome this, a weighted numerical score, based on the stopping criteria, was devised to capture shifts in severity between challenges.

Development of the severity score

An ideal scoring system for allergic reaction severity should be based on easily and routinely recorded variables. The score ideally should be applicable to all types of allergic reactions involving all triggers and to all patient populations. A severity classification should reflect two concepts: i) as the severity increases the number of involved organ systems usually increases and ii) cardiovascular, neurological, bronchial and laryngeal involvement are potentially life threatening and therefore signify more severe reactions.²²⁶ Numerical weighting was assigned by a consensus panel comprising a committee of experts. All

symptoms across all organ systems in the stopping criteria were ranked from least severe to most severe. Each symptom was assigned a weight such that a combined total score of all green symptoms could not add up to a greater value than a single yellow symptom, with the same reasoning adopted for yellow symptoms and red symptoms. In previous fatal anaphylaxis enquires the mode of death has been reported to be respiratory arrest.¹⁴¹ In another fatal anaphylaxis study by Mullins et al, acute dyspnoea was noted in 64% cases.¹⁵⁹ Low et al reported that cardiac and peripheral vascular symptoms were the leading cause of death in fatal anaphylaxis cases.²²⁷ Cardiovascular and respiratory symptoms have previously been considered by consensus to be life-threatening features of anaphylaxis.²²⁸ Therefore severe cardiovascular and respiratory symptoms were deemed potentially “life-threatening red symptoms” and were assigned a considerably higher weight than all other symptoms reflecting their importance. The score was road tested on example cases to ensure that there was adequate discrimination between shifts in severity particularly at the mild/moderate end of the spectrum where shifts may be smaller. The score is shown in Table 13.

Table 13: Weighted numerical severity score

Numerical score	Symptom
1	Pruritus -Occasional scratching [Green]
1	Rash- Few areas of faint erythema [Green]
1	Rare bursts of sneezing occasional sniffing [green]
1	Transient Nausea [Green]
1	Throat tingling/altered sensation in throat [Green]
1	Oral itching [Green]
1	Itching in inner ear canal [Green]
2	Pruritus- scratching continuously for >2 mins at a time [Green]
2	Transient abdominal pain[Green]
2	Chest tightness without any fall in PEFr [Green]
6	Rash- Areas of erythema [Yellow]
6	I Bursts < 10, intermittent rubbing of nose, and/or eyes or frequent sniffing [Yellow]
6	Continuous rubbing of nose and/or eyes [Yellow]
6	Hard continuous scratching>excoriations [Yellow]
10	Persistent nausea[yellow]
12	Urticaria-<3 hives or mild lip oedema [Yellow]
12	> 3 discrete episodes of throat clearing or cough [Yellow]
13	Chest tightness with less than or equal to 10% fall in PEFr [Yellow]
15	Persistent abdominal pain[yellow]
18	Emesis/diarrhoea (1 episode) [Yellow]
20	Periocular swelling and/or long bursts of sneezing, [Yellow]
20	Persistent rhinorrhoea [Yellow]
20	Urticaria <10 or >=3 hives or significant lip or face oedema [Red]
20	Altered level of consciousness [Red]
25	Persistent throat tightness [Yellow]
25	Weak/dizzy or tachycardia [Yellow]
25	Emesis /diarrhoea (more than 1 episode) [Red]
30	Rash- Generalised marked erythema>50% [Red]
30	Urticaria-generalised involvement [Red]
35	Chest tightness with a10% - 20% fall in PEFr [yellow]
40	Hoarseness or frequent dry cough [Red]
50	Expiratory or inspiratory wheeze [Red]
70	Chest tightness with a > 20% fall in PEFr[red]
70	Use of accessory muscles [Red]
100	Stridor [Red]
150	Drop in BP of >20% from baseline [Red]
200	Cardiovascular collapse/signs of impaired circulation [Red]

Assessing the change in severity within an individual as a result of repeated challenges and co-factors

A threshold was called at the development of objective symptoms by the investigator and therefore participants' reactions were unable to evolve fully. As a result of this there is an unavoidable endogeneity between threshold dose and severity in this study. In order to overcome this problem a consistent dose across all challenges within an individual was examined for severity. The chosen dose was the highest dose reached in all challenges (i.e. equivalently, the *lowest* threshold dose reached *across* the 4 challenges) as demonstrated in Table 14.

Table 14: Method for determining comparable dose in two example participants

Example participant 1	Challenge type	Threshold dose reached	Dose chosen for Comparison
	Baseline	6 (100mg)	5 (30mg)
	Exercise	5 (30mg)	
	No intervention	6 (100mg)	
	Sleep	5 (30mg)	
Example participant 2	Baseline	8 (1000mg)	6 (100mg)
	Exercise	6 (100mg)	
	No intervention	7 (300mg)	
	Sleep	6 (100mg)	

To estimate the within-participant impact of repeat challenges on severity, a linear fixed effects model of severity was estimated on i) a linear trend representing visit number by participant and ii) a set of dummy variables representing visit number by participant (i.e. visit 2, visit 3 and visit 4). The marginal impact of visit number on severity was expressed relative to severity on visit 1.

Although exercise and sleep deprivation interventions were randomised for each participant by visit number, they were never performed on visit 1, which was used to establish a baseline without intervention. Therefore, it was important to control for the impact of interventions on the average severity of visits 2, 3 and 4 relative to visit 1 by including dummy variables for interventions by challenge.

Frequency of symptoms

The frequency of each symptom across all challenges was reported. The change in symptom frequency (arranged in systems) with sleep deprivation and exercise compared to baseline challenge was reported.

Using a fixed effects regression analysis, I examined at a system level whether there was a change in symptom severity with sleep deprivation and exercise.

Reproducibility of symptom patterns within and across individuals

Participants' symptom patterns during each challenge were compared. A comparison of symptom patterns across participants was also examined. A bioinformatics technique called pairwise sequence alignment was used. This has previously been used to align two query sequences of amino acids to find similarities in two proteins.²²⁹

Using the same method as described above, for each individual, the lowest dose common to all challenges was taken and all symptoms up until that point in each reaction were observed. If a symptom occurred twice it was counted once only.

To compare sequences, a 37 (all possible symptoms) digit binary code was produced for each reaction based on the presence (1) and absence (0) of each symptom. The percentage match for pairs of reactions was calculated based on the coincident presence or absence of each symptom. Percentage match was identified by identifying the number of matches in the pair divided by the length of sequence (i.e. 37 symptoms) and multiplying by 100.

In order to assess across-participant symptom patterns, 1000 randomly selected pairs of reactions across-participants were selected and analysed for pairwise matches.

Predictors of severity

Using linear regression, the severity of reaction was correlated to clinical characteristics including: presence of asthma, presence of eczema, age of onset of peanut allergy, severity of worst historical reaction, number of previous adverse reactions, and the timing of last community reaction. Severity was also correlated to markers of sensitisation including SPT wheal to peanut (mm), specific IgE peanut and specific IgE to Ara h2.

Analysis of anaphylaxis group

Participants who suffered from anaphylaxis during any of their challenges were analysed in a separate group.

For the purposes of this study anaphylaxis was defined as two or more of the following rapidly occurring symptoms (minutes to up to 2 hours) following exposure to peanut: generalised involvement of the skin-mucosal tissue, respiratory compromise (pronounced dyspnoea, wheeze, bronchospasm), reduced blood pressure or associated symptoms, severe and persistent abdominal symptoms (cramping abdominal pain and vomiting).²³⁰

Univariate linear regressions of the presence of anaphylaxis on the underlying characteristics (linear probability model) were used to compare whether the underlying characteristics were more predictive of developing anaphylaxis compared to not developing anaphylaxis.

Participants who suffered life threatening anaphylaxis will be reported separately as clinical case summaries at the end of the results section.

Adrenaline use

The frequency of intramuscular and nebulised adrenaline use was reported.

Severity of symptoms in relation to dose

The frequency of severe symptoms grouped by organ system in relation to dose was examined.

Reporting of late symptoms

Immediate symptoms were defined as symptoms during the challenge or within 2 hours of the last challenge dose. Late-onset symptoms were defined as symptoms occurring more than 2 hours after the last challenge dose. These symptoms were reported in two groups- symptoms which recurred after apparent resolution of the initial phase of symptoms, termed ‘separate resurgence’ or those which occurred as part of a protracted reaction.

Perception of severity

Both the investigator and participant were asked to subjectively appraise their allergic reactions. In order to ensure that the results were not presented in an arbitrary manner and that they were statistically documentable and analysable, a visual analogue scale (VAS) was developed. Visual analogue scales have been used in the past for a multitude of disorders including allergic rhinitis²³¹ and angioedema.²³² A 10cm long horizontal line was produced

with verbal descriptors at each end to express the extremes of severity. A scale of 0 to 10 was selected. The participant was asked to place a cross on the straight line to express their assessment. Using this scale, symptoms in each organ system were scored. The participant was also asked to give an overall reaction severity score (Appendices 4 and 5). Finally the points from each organ system and the overall severity score were added to give an aggregate points score. Both the overall reaction severity score and aggregate score were analysed. Both the investigator and participant score were correlated with each other. For the strength of correlation, the Pearson correlation coefficient was computed. A correlation of greater than 0.5 was considered a strong correlation, 0.3-0.5 a moderate correlation and less than 0.3 a weak correlation.

RESULTS

Participants

Fifty seven participants mean age 21 years (range 18-39) were included in this analysis. All participants received a baseline challenge. Two participants had a life-threatening reaction at their baseline challenge and did not proceed in the study. In total, 187 active peanut challenges were performed. Eleven participants did not proceed to the intervention stage following baseline challenge, forty-one participants completed all 4 active challenge days and 46 participants completed at least 1 intervention challenge. A distribution of the overall challenge severity score is shown in Figure 8 and as a comparison in Table 15, the distribution of severity grades according to Sampson.¹⁵³ The mean of the sample was 69.2 and standard deviation 31.9.

Figure 8: Distribution of overall challenge severity score for all challenges (n=187)

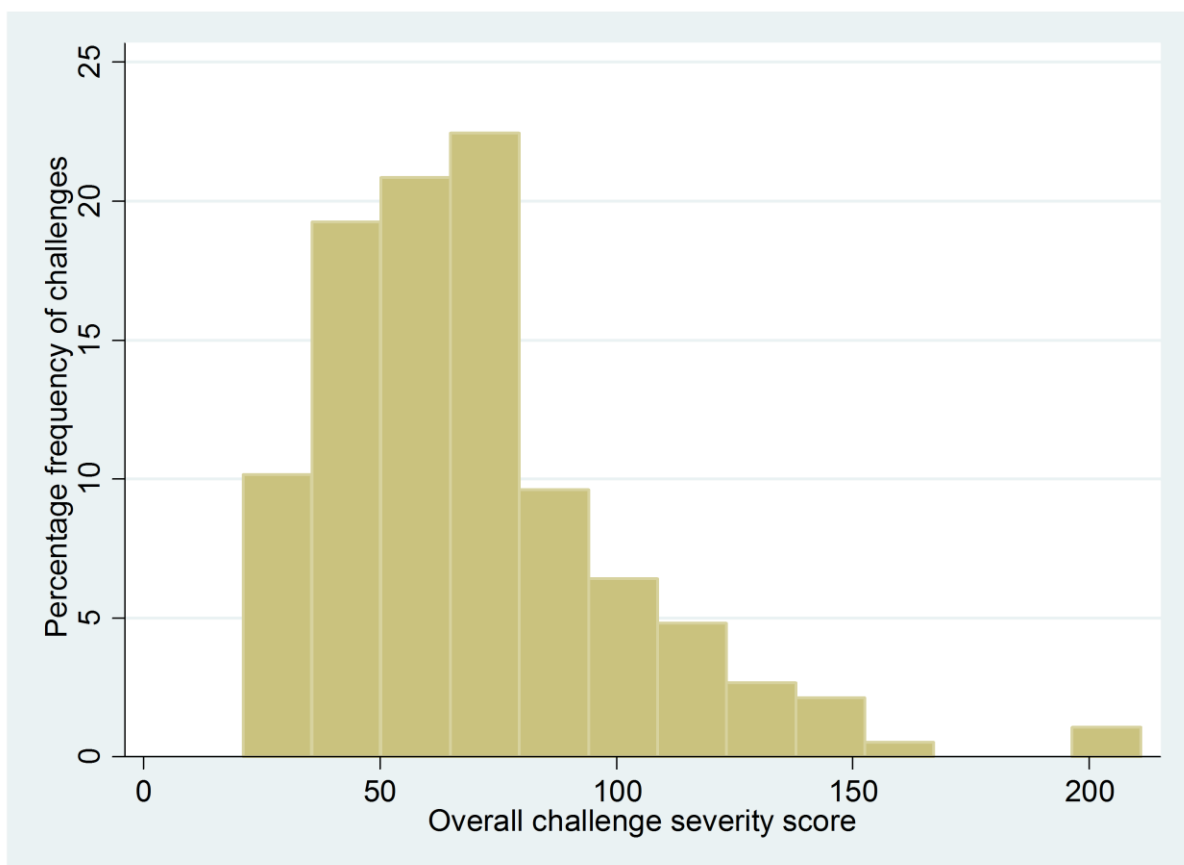


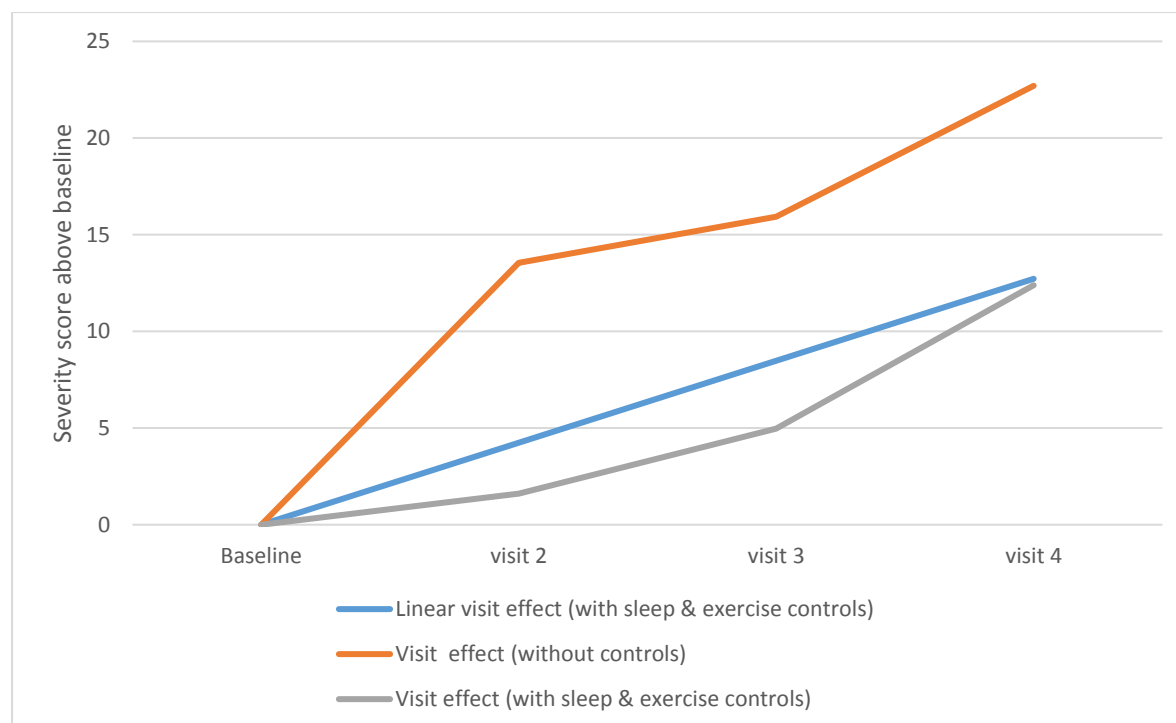
Table 15: Distribution of severity grades according to Sampson Classification

Severity grade	1	2	3	4	5
Baseline	0	30%	44%	26%	0
Exercise	0	32%	43%	25%	0
No intervention	0	35%	46%	19%	0
Sleep	0	38%	43%	19%	0

Assessing the change in severity within an individual as a result of repeated challenges

Estimating within-participant using a linear fixed effects regression model based on 176 challenges across 46 participants (the 11 participants with only one observation being omitted, and each included participant typically undergoing 4 challenges), a positive effect of visit number on severity at constant dose was observed (Figure 9).

Figure 9: Within-participant visit effect on challenge severity (at constant dose)



When examining the within-participant effect of visit number using dummy variables for visits 2, 3 and 4 (column 1 in Table 16) a strong monotonic increase by each progressive visit was observed at a highly statistically significant level. However, exercise and sleep

deprivation interventions were only ever implemented on visits 2 3 or 4, which would tend to bias the coefficients on the visit dummies upwards and bias the p-values downwards.

When controlling for exercise and sleep, although the incremental impact remains monotonic, disentangling the impact of exercise and sleep imposed a cost to the statistical significance of these coefficients (column 2 in Table 16).

Estimating the effect of each progressive visit as a linear trend rather than using visit dummies whilst still controlling for exercise and sleep produces a statistically significant effect of visit number on severity (column 3 in Table 16). Given an average severity score across all participants of 25.8 on visit 1, each subsequent visit added 4.2 points on severity.

Table 16: Summary of results of three regression estimations, illustrating the within-participant effect of visit number on severity – using visit dummies with and without exercise and sleep controls (1 and 2), and a linear visit trend with exercise and sleep controls (3).

	(1) Within-participant severity	(2) Within-participant severity	(3) Within-participant severity
<i>Visit 2</i>	13.54* (0.067)	1.605 (0.836)	
<i>Visit 3</i>	15.93*** (0.009)	4.971 (0.510)	
<i>Visit 4</i>	22.72*** (0.002)	12.39* (0.071)	
<i>Exercise</i>		12.51* (0.056)	11.37* (0.062)
<i>Sleep</i>		20.53*** (0.002)	19.25*** (0.003)
<i>Linear trend</i>			4.238* (0.051)
<i>Constant</i>	30.94*** (0.000)	31.00*** (0.000)	25.80*** (0.000)
<i>Participant fixed-effect</i>	Yes	Yes	Yes
<i>N</i>	176	176	176
<i>Participants</i>	46	46	46

Robust standard errors, *p*-values in parentheses

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$

Assessing the change in severity within an individual as a result of interventions (sleep deprivation and exercise)

Using a linear fixed effects model to estimate within-participant effect controlling for visit number using visit dummies a positive effect was seen, with both interventions increasing the severity of reaction (column 2 Table 16 ; the effect was robust to controlling for the visit effect using a linear trend instead of visit dummies, see column 3 of Table 16). The effect was larger in sleep deprivation. Sleep was associated with a 20.5 (0.67 SD) points higher reaction severity ($p=0.002$) compared to exercise which was associated with a 12.5 (0.39 SD) higher points severity ($p=0.056$).

Frequency of symptoms experienced by participants during challenge and how these change with sleep deprivation and exercise.

The frequency of the 37 symptoms during all challenges is displayed in Figure 10. Symptom frequency at a system level for baseline challenges and no-intervention combined is shown in Figure 11. Cutaneous symptoms were most common, followed by throat symptoms. Changes occurring in symptom frequency with sleep deprivation and exercise are shown in Figures 12(a) and (b) respectively. Only small changes in symptom frequency were observed for sleep deprivation. The most noticeable effect on symptom frequency was observed in exercise where cardiovascular symptoms increased by 21% and rhinitis symptoms by 13%.

Figure 10: Frequency of symptoms during all 187 challenges

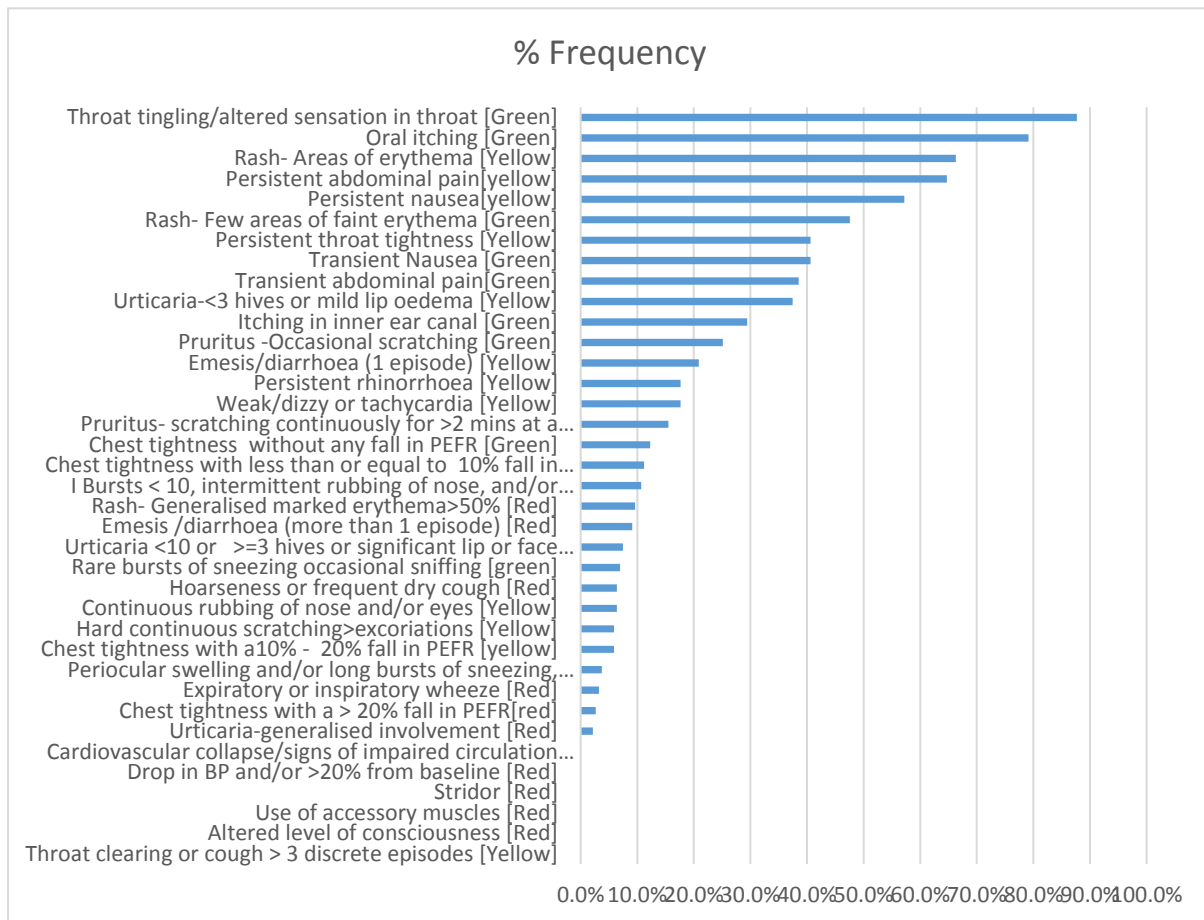


Figure 11: System involvement during baseline and no intervention challenges (n=100).

Cutan, Cutaneous; *Gastro*, Gastrointestinal; *Rhin*, Rhinitis; *Resp*, Respiratory; *Cardio*, Cardiovascular

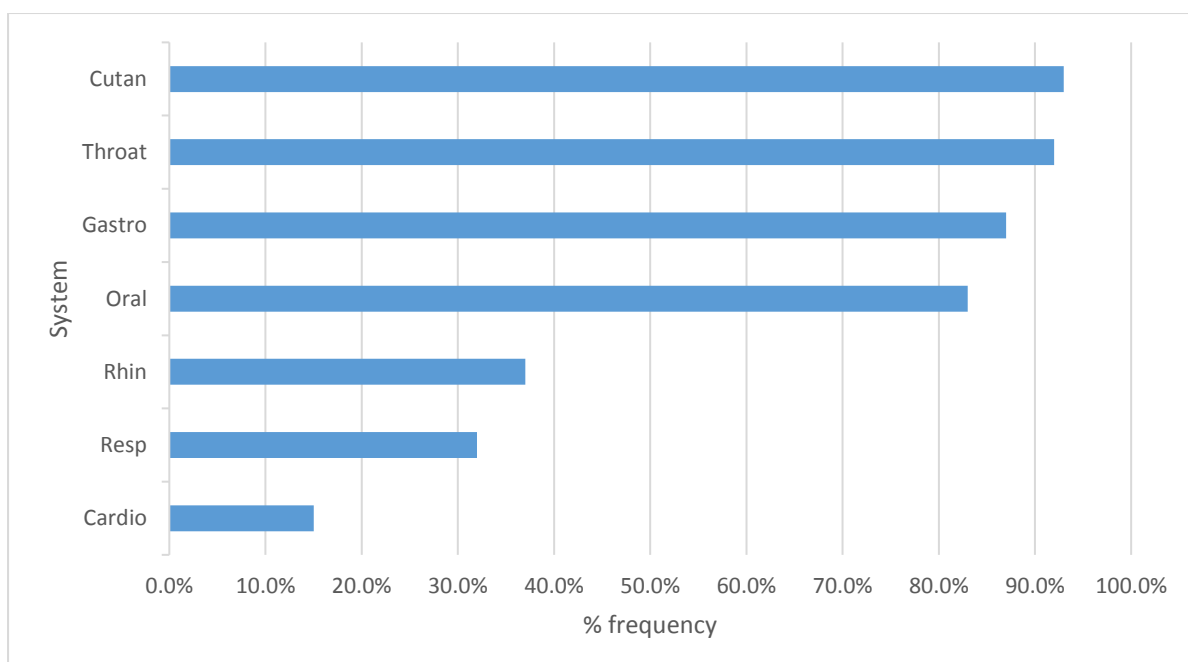


Figure 12(a): % change of system involvement frequency with sleep deprivation (n=43).

Cutan, Cutaneous; *Gastro*, Gastrointestinal; *Rhin*, Rhinitis; *Resp*, Respiratory; *Cardio*, Cardiovascular

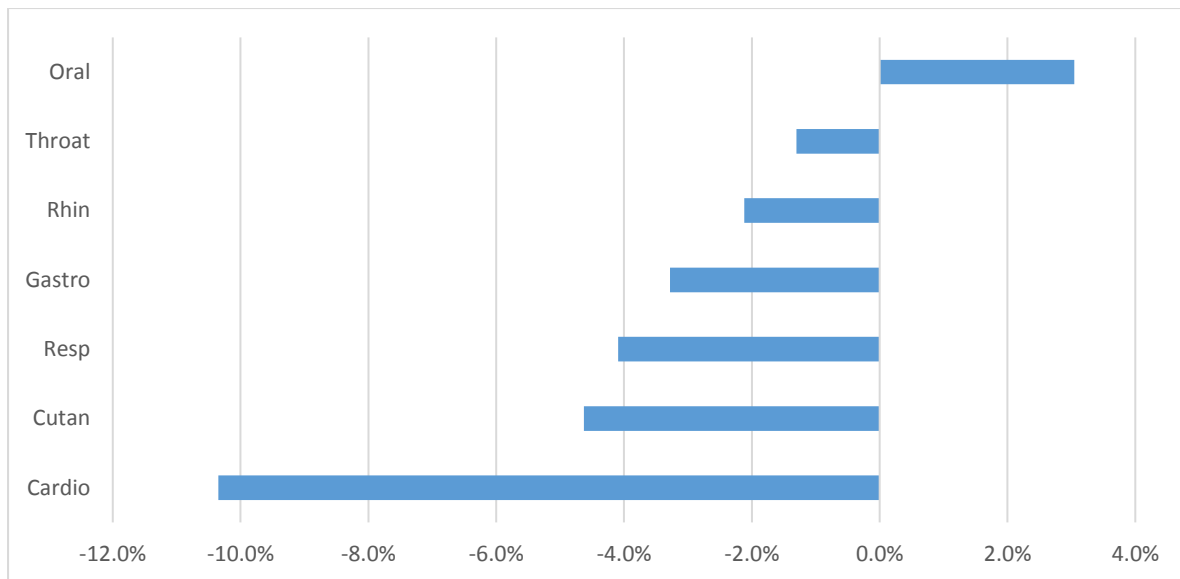
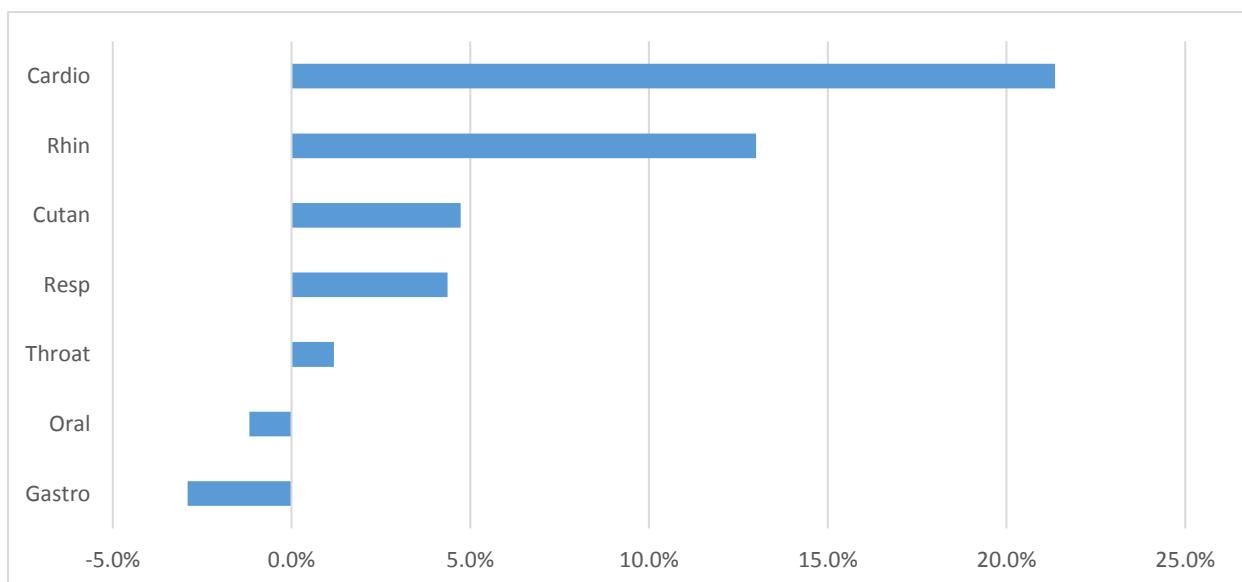


Figure 12(b): % change of system involvement frequency with exercise (n=43).

Cutan, Cutaneous; *Gastro*, Gastrointestinal; *Rhin*, Rhinitis; *Resp*, Respiratory; *Cardio*, Cardiovascular



How does the severity of symptoms change at a system level with exercise and sleep deprivation?

A fixed effects model was used to examine within-participant changes in severity at system level with sleep deprivation and exercise. Significant effects were only observed in sleep

deprivation challenges for 2 systems. Exercise did not produce any significant effects on increasing symptom severity at system-level. The severity of gastrointestinal and throat symptoms increased under sleep deprivation conditions by 5.8 (0.55 SD) ($p=0.036$) severity points and 7.0 points (0.75 SD) ($p=0.003$) respectively.

Persistence of participant symptom pattern within individuals with repeated challenges.

There were 304 possible within-participant pairs of reactions. The average pairwise percentage match was 81.9%

Persistence of symptom patterns across individuals

1000 across-participant pairs of reactions were selected ensuring that reactions were compared with like for like doses. The average pairwise percentage match was 78.3%.

Correlation of reaction severity with participant characteristics

There was a positive correlation of SPT wheal size with severity of reaction ($p=0.03$). Presence of asthma, eczema, age of onset of peanut allergy, severity of worst historical reaction, number of previous adverse reactions, timing of last community reaction, specific IgE peanut and specific IgE to Arah 2 could not be correlated.

The effect of asthma on severity

Although the presence of asthma was not associated with a higher reaction severity overall, the presence of asthma was associated with a higher severity of respiratory symptoms only ($p=0.02$). When examining the whole sample (including challenges with no respiratory symptoms/zero respiratory severity) the presence of asthma was associated with an increase of 7 points on the respiratory severity scale (0.33SD) ($p=0.02$). Using only the challenges with respiratory symptoms the presence of asthma was associated with a 16 point increase in respiratory severity (0.74SD) ($p=0.017$).

Anaphylaxis group

Out of the 187 allergic reactions induced in the study, 29 episodes were anaphylaxis (16%). These episodes occurred in 13 participants. Two participants were excluded at baseline due to severe anaphylaxis. Of the remaining eleven participants who proceeded to the intervention stage, 64% developed a repeat episode of anaphylaxis. When examining whether the underlying clinical characteristics described above previously were more predictive of anaphylaxis compared with non-anaphylaxis a positive correlation with severity of historical

reactions ($p=0.001$) and history of adrenaline use ($p=0.001$) was seen. Anaphylaxis was less likely with higher peanut IgE ($p=0.05$), higher Arah2 ($p=0.005$) and less likely with asthma ($p=0.029$). Univariate analyses did not show significant associations of any of the other candidate predictors including presence of eczema, age, age of onset of peanut allergy or number of adverse reactions on the likelihood of anaphylaxis.

Frequency of adrenaline use

The overall rate of adrenaline use in the study was 15%. Three participants required two doses of intramuscular adrenaline to stabilise. The most frequent reason for adrenaline administration was to correct respiratory symptoms, $n=22/28$ (79%).

The frequency of adrenaline use is shown in Table 17.

Table 17: Frequency of intramuscular (IM) and nebulised adrenaline administration.

	Baseline	Exercise	Sleep	No intervention	Total
Number of times IM adrenaline administered	10/57	7/44	6/43	5/43	28/187
%	17	16	14	12	15
Number of times nebulised adrenaline administered	3/57	0	0	0	3/187
%	5				2
Number of times 2 doses of IM adrenaline administered	3/57	0	0	0	
%	5				2

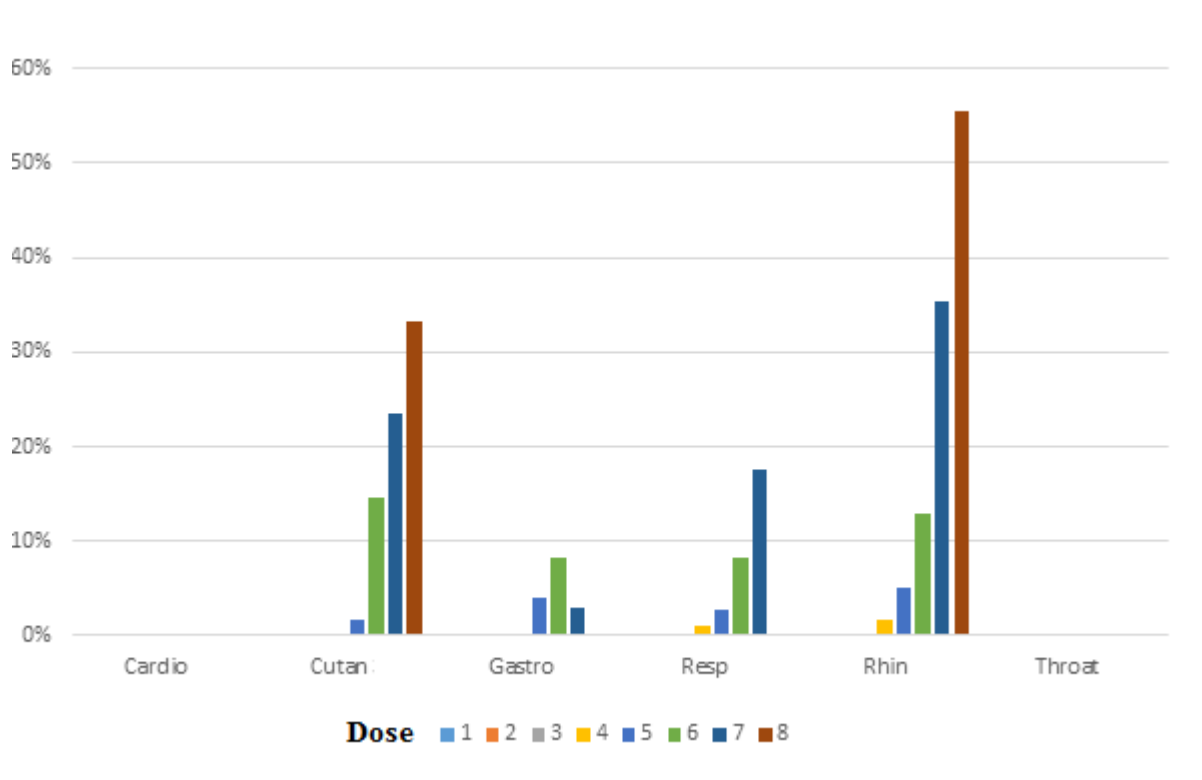
Participant and investigator perception of severity

There was a moderate correlation between the investigator and participant scores for both Overall Reaction Severity, 0.39 ($p < 0.05$) and Total VAS score, 0.44 ($p < 0.05$). A better (strong) correlation was observed when within-participant VAS scores were compared: Overall Reaction Severity, 0.35 and Total VAS score, 0.62 ($p < 0.05$). Although there was a strong correlation between the investigator VAS score (0.52 $p < 0.05$) and the overall numerical severity score for the challenge, the patient scores were poorly correlated (0.27 $p < 0.05$).

Relationship of dose to severity of symptoms by system

A summary of the frequency of severe symptoms of each system occurring at each dose is shown in Figure 13. Severe cardiovascular symptoms never occurred. The overall frequency of severe respiratory symptoms increased as dose increased but remained uncommon. The most common severe symptoms were severe cutaneous symptoms and severe rhinitis symptoms which occurred at Dose 8.

Figure 13: Percentage of severe symptoms as a % of challenges occurring at that dose



Reporting of late symptoms

Symptoms which developed more than 2 hours after the last dose are shown in Table 18. Delayed symptoms occurred in 7/187 (4%) reactions and all occurred between 2-2.5 hours of the dose. Cutaneous symptoms were the most common delayed symptom (6/7 reactions, 86%). However delayed respiratory symptoms also occurred in 1/7 cases (29%). One participant had delayed symptoms on 3 separate occasions.

Table 18: Nature of delayed symptoms and treatment administered. Symptoms classed as separate resurgence if symptoms appeared after an initial resolution.

Participant	Symptom	Time from last dose (mins)	Treatment	Protracted or separate resurgence
1	Pruritus (gen.)	129	Cetirizine	Separate resurgence
2	Erythema (gen.)	122	Adrenaline IM	Separate resurgence
3	Hoarseness and cough/Erythema (gen)	130	Adrenaline IM/IV fluids	Protracted
4	Abdominal pain	121	Cetirizine	Separate resurgence
5	Urticaria (gen.)	150	Cetirizine	Separate resurgence
5	Urticaria (gen.)	121	Adrenaline IM	Protracted
5	Urticaria (gen.)	124	Adrenaline IM	Protracted

Case summaries of two excluded participants

Participant 1

18 year old male diagnosed with peanut allergy aged 2 after eating a bite of peanut butter on toast. He had suffered two previous reactions which were mild, developing erythema and vomiting only. No co-morbid conditions including asthma present.

Attended for baseline challenge and was well on the day of challenge.

During doses 1, 2, 3 and 4, no symptoms

Following dose 5 he developed mild oropharyngeal symptoms and transient throat tightness lasting for 3 minutes only and resolving completely.

Following dose 6 (cumulative dose 133.3mg peanut protein) participant started developing abdominal pain 23 minutes after dose which increased in severity. Seventeen minutes after the onset of abdominal pain participant started to feel very lightheaded with worsening abdominal pain. Participant became hypotensive BP dropped to 107/65 from a baseline of 125/71 which had been performed 15 minutes before the onset of symptoms and also

mounted a tachycardia (HR106). At this point adrenaline IM (0.5mg) was administered. However he then started to become vacant and eyes rolled back. After laying the participant down he developed almost immediate profuse projectile vomiting followed by widespread generalised erythema (IV hydrocortisone and IV chlorpheniramine given). Participant started to recover but then became aware of his own heart beat beating irregularly. ECG performed which showed junctional atrial ectopics. This settled after about 30 minutes- repeat ECG showed sinus rhythm but tachycardia still present, probably post adrenaline administration. There were delayed symptoms with the participant developing a frequent dry cough (61 minutes post dose) and worsening erythema (69 minutes post dose). At this point given further IM adrenaline 0.5mg IM (with regular monitoring of HR, BP and ECGs), IV fluids, salbutamol nebulised (5mg) and high flow oxygen 15L via non-rebreathe bag. Oxygen saturations were maintained throughout. Participant was too unwell to provide a peak flow but good symptomatic response following second lot of adrenaline. Eventually settled after a further hour. Participant felt well enough to go home, was offered admission but declined.

Using the Visual Analogue Scale the participant graded his overall reaction severity 6/10 with a total aggregate score of 23/70, compared to my score as the investigator of 9/10 for overall reaction severity and a total aggregate score of 53/70.

The participant was keen to return to continue the study but we decided to exclude him given the severity of his reaction.

Participant 2

23 year old female diagnosed with peanut allergy at the age of 2 after consuming one peanut and developed cutaneous symptoms solely. She had only suffered 1 adverse reaction previously. Participant had a background history of widespread but well controlled eczema and no asthma.

During dose 1,2,3,4 she developed no symptoms.

Following dose 5 she developed oropharyngeal tingling and started rubbing her hands together and scratching the eczema on her hands. Her partner (who was present at the time) reported the latter (check) was habitual.

After dose 6 she developed abdominal pain 11 minutes after dose along with nausea. She then started to develop urticaria on her abdomen 18 minutes after dose. At that point she was given IV hydrocortisone and chlorpheniramine. However, at 33 minutes post dose the

erythema became generalised and IM adrenaline 0.5mg was given. At 50 minutes post dose started to develop a frequent dry cough. Further IM adrenaline given 8 minutes later. Also given nebulised salbutamol (5mg) at that point. There was a limited response to this and at 78 minutes post dose she developed generalised polyphonic wheeze. Given nebulised adrenaline (1mg/5ml N. saline 0.9%). However, she did not improve and developed recurrent chest tightness with use of accessory muscles and began to desaturate on nebuliser (to 92%) so was given 2 further lots of nebulised adrenaline interspersed with back to back salbutamol nebulisers (5mg). The critical care outreach team was alerted and they reviewed the participant, by which point she had started to improve. They did not add further treatment. Participant had continuing pruritus and worsening erythema at 88 minutes post dose so was given oral cetirizine and prednisolone 30mg. Participant was maintained on high flow oxygen 15L via non-rebreathe bag and IV N saline 1 litre. Her BP was maintained throughout. She tolerated IM adrenaline well with max heart rate of 134. Made a good recovery after 1-2 hours.

Using the Visual Analogue Scale the participant graded her overall reaction severity 8/10 with a total aggregate score of 37/70, compared to my score as the investigator of 9/10 for overall reaction severity and a total aggregate score of 46/70.

DISCUSSION

In this study, the effect of repeated challenges within an individual results in reactions of increasing severity with each visit. Separately, co-factors such as sleep deprivation and exercise can increase the severity of peanut allergic reactions. In particular sleep deprivation increased the severity of gastrointestinal symptoms. For the first time, I show that symptom patterns during peanut allergic reactions are homogenous within individuals and also that commonality exists in symptom patterns across participants in our sample. Limited correlations were found between clinical characteristics and severity. Skin prick test size was positively correlated with reaction severity but other markers of sensitisation such as peanut or Arah2 IgE were not. A history of severe reactions and adrenaline use was correlated with the presence of anaphylaxis during challenge. Furthermore the presence of asthma was correlated with a significant increase in severity of respiratory symptoms if respiratory symptoms were present in the reaction. Lastly using a novel visual analogue scale for measuring the participant's perception of severity, a poor correlation was observed between the participant's perception of the reaction and the overall numerical severity score.

Assessing the change in severity within an individual as a result of repeated challenges

In this study, when a linear model was fitted to the reaction severity at each challenge, the severity at visit 4 was significantly higher than that at visit 1 by 12 points. There are limited data on how reaction severity changes over time within an individual with most information coming from patients' accounts of reaction. Glaumann *et al* found no association between severity scores at two consecutive challenges in 14 peanut allergic patients.¹²⁶ Separately Van der Leek *et al* followed up children with peanut allergy and compared index reactions with subsequent reactions following accidental exposure and found that 44% patients who had suffered a non-life threatening reaction initially would subsequently suffer from life threatening symptoms.²³³ However there were no challenge data in this study and community reactions will inevitably differ in dose exposure which will endogenously influence severity. In our study, even with controlling for dose an effect of increasing severity was still observed. Three month intervals were planned in the study to guard against the possibility of a desensitisation effect. However, this severity analysis has shown that the opposite effect may be happening, that patients may develop increased reactivity at each subsequent exposure. Furthermore caution must be exercised in viewing severity as a linear function of visit number given the observed non-linearity of the coefficient on the visit dummies. Such a

distinction may prove important medically as a nonlinearly rising effect might imply cumulative risk of a very severe reaction following a number of repeated reactions even more severe than the proposed linear 4 point increase with each challenge.

Assessing the change in severity within an individual as a result of interventions (sleep deprivation and exercise)

Both sleep deprivation and exercise increase overall reaction severity with sleep deprivation having a greater effect than exercise. As discussed in the previous chapter it is likely that exercise may be directly increasing severity by a greater availability of allergen in the systemic circulation. Exercise leads to splanchnic hypoperfusion, possibly inducing a relative gut ischaemia causing tight junction dysfunction. A possible increased liberation of allergen into the blood stream may then ensue through an increase in paracellular transport.¹³⁷ Another possible contributory factor that I noticed during the exercise challenge compared to the other challenges, is that often participants did not report many of the early warning signs such as oropharyngeal pruritus, altered throat sensation or transient abdominal pain which occurred soon after the dose was given. Perhaps this occurred because participants were distracted whilst undertaking the exercise period or there may have been a degree of endogenous adrenaline release during the exercise period compensating for the early symptoms which may have resulted in delayed recognition and symptom reporting by participants. It may be that this endogenous adrenaline release and partial compensation is responsible for the smaller effect seen during exercise compared to sleep deprivation. As postulated in the previous chapter, it is possible that acute sleep loss, which is a stress-inducing stimulus, leads to increased activation of the brain-gut-axis and propagation of mast cell activation and the allergic response inducing an increased gut permeability.²³⁴ Indeed the results of this study indicate that the severity of gastrointestinal symptoms significantly increases during sleep deprivation which supports this hypothesis. Brown et al have demonstrated a correlation between GI manifestations and the severity of anaphylaxis.²³⁵ Furthermore the presence of chronic and relapsing GI symptoms and the degree of gastrointestinal permeability positively correlated with the severity of anaphylaxis in humans.²³⁶

Frequency of symptoms experienced by participants during challenge and how these change with sleep deprivation and exercise.

Cutaneous and throat symptoms were the most frequently occurring during baseline and no-intervention challenges (93% and 92% respectively), followed by gastrointestinal symptoms

87%. Reassuringly, severe throat symptoms during challenge such as stridor or dysphagia were rare and the majority of throat symptoms were due to an altered sensation in the throat or persistent throat tightness. These results differ from analyses which examine the frequency of anaphylaxis due to all causes. Beyer et al observed in their series looking at anaphylaxis of all causes in the community that 86.1% reactions involved symptoms of the cardiovascular system, in this study we observed cardiovascular involvement in 17.6% of all challenges.²³⁷ Cianferoni *et al*'s series of 983 children who underwent OFC to egg, milk and peanut, had a lower incidence of cutaneous involvement (56%) and gastrointestinal involvement (59%).²³⁸

When examining changes in system involvement with sleep and exercise, only minor changes in symptom frequency were observed during sleep deprivation. However, conversely with exercise, there was a notable increase in cardiovascular symptoms (21.4%). Cardiovascular symptoms manifested themselves predominantly as dizziness and physical weakness. These symptoms were out with the normal physiological responses during exercise. During exercise, 80% of cardiac output is diverted to active skeletal muscles at maximal rates of work.²³⁹ Furthermore during exercise there is a decrease in total peripheral resistance which is balanced by an increased cardiac output and mean arterial pressure. However during an allergic reaction, as a result of mast cell mediator release, peripheral vasodilatation may occur, exacerbating the already reduced peripheral resistance produced by exercise. This may result in an inadequate cardiovascular compensatory response leading to symptoms of dizziness, weakness and tachycardia.

Factors associated with increasing reaction severity

The only significant positive association found in this study was that of SPT wheal diameter to peanut and overall reaction severity score. Other markers of sensitisation including specific IgE to peanut and Arah2 could not be correlated. A previous study of community reactions based on patient reported symptoms, using a simpler score, found no link between peanut SPT size and severity grade.¹²² The association between higher levels of peanut-specific IgE and reaction severity has previously been described in a study by Neumann-Sunshine *et al* in a study of 782 patients with persistent peanut allergy.²⁴⁰ Previously it has been shown in the EuroPrevall cohort that although Arah2 confers an extremely high probability of a systemic reaction (97%), no significant relationship to reaction severity during food challenge was observed, necessitating more prospective research.⁶³ The relationship between IgE binding to allergen and reaction severity is likely to be a more complex picture with a more relevant

factor being the promiscuity of epitope binding than rather than the recognition of individual proteins²⁴¹ in combination with IgE affinity and avidity.

Symptom pattern

Little data exist on the pattern of symptoms and the sequence of symptom progression both within and across allergic individuals. Using a sequence alignment technique, homogeneity of symptom patterns within an individual could be demonstrated. However, reaction patterns across individuals in our sample were almost as similar (78.3% across versus 81.9% within). This analysis looked at absolute numbers of all symptoms which appeared over the course a reaction however further work is needed on the combinations of symptoms which appear during a reaction and whether there are tendencies for certain symptoms to co-appear. However the information presented here will allow clinicians to better inform patients about the likely nature of further reactions in terms of organ systems involved.

Adrenaline use

The overall rate of intramuscular adrenaline use in this study was 15% and nebulised adrenaline 2%. This is a fairly comparable rate to other studies examining the frequency of adrenaline administration during oral food challenge. Yanagida et al reported a rate of 23% for IM administration in patients undergoing oral food challenges prior to the commencement of immunotherapy. The rate of use of inhaled adrenaline was 13%.²⁴² In contrast, Noone *et al* reported a much higher rate of adrenaline use in their study, again screening subjects for food therapeutic trials.²⁴³ In their study, intramuscular adrenaline was administered in 39.2% cases, however the higher rate may be accounted for by differences in physician practice for example, the use of adrenaline to treat severe abdominal cramping which was not an indication in the TRACE study protocol. The incidence of multiple doses of IM adrenaline administration was only 2% (3/187 challenges). The first participant requiring two doses was one of the first participants to undergo the study and developed a resurgence of cutaneous symptoms at a delayed interval. This was the first time that this phenomenon had been witnessed and as a result a second dose of adrenaline was administered as a precaution to terminate the reaction. The other two participants were poorly responsive to the first dose and had protracted reactions (described separately). In food challenge studies the rate of multiple doses ranges from 0.68-6.5%.^{242,244} Of course studies focussed on community reactions presenting to the ED department report higher rates of repeated epinephrine use 13-16%.^{245,246}

Asthma as a risk factor

The presence of asthma is considered to be a risk factor for fatal anaphylaxis. Moreover Summers et al report a large association between asthma and acute bronchospasm after the ingestion of nuts.²⁴⁷ In a European anaphylaxis registry asthma or underlying bronchial hyperreactivity was identified as a possible risk factor.²⁴⁸ In this study the presence of asthma was not correlated with a higher overall reaction severity. However when a different question was posed as to whether the presence of asthma increased the severity of established respiratory symptoms only then asthma did serve as a risk factor in increasing severity. It is possible that this occurs as a result of active inflammatory processes within the airways and reduced physiological reserve in patients with asthma who develop respiratory symptoms during reaction.²³⁵ Less physiological reserve may mean that asthmatics progress to a severe state more quickly, particularly if their asthma is less well controlled. In our study all patients had well controlled asthma, nonetheless an effect was still observed. These findings contrast with Van Erp's group who compared asthmatics to non-asthmatics and found that in children with a positive food challenge, asthmatics did not report a severe respiratory reaction more commonly (29% vs 22% $p=0.50$). They also found that asthmatics did not have a reaction with involvement of the lower airways during challenge more frequently.²⁴⁹ However other studies support the importance of asthma as a risk factor with one study reporting that in patients with severe asthma the risk of life threatening bronchospasm during nut associated anaphylaxis was increased 6.8 fold although this relative risk was only 2.7 times higher for the patients with mild asthma.²⁵⁰ Thus when managing patients with food allergy the role of asthma in increasing the severity of respiratory symptoms should they develop should not be underplayed and patients should be well versed on optimisation of their general asthma control.

Anaphylaxis

Fifteen percent of reactions met the criteria for anaphylaxis. This is in line with other studies which estimate an incidence of anaphylaxis of between 10-15% in patients challenged to peanut.²⁴⁹ In our study these reactions frequently occurred repeatedly within one individual, 64% patients suffered from a repeat episode of anaphylaxis. Reisman analysed the incidence of repeat sting induced anaphylaxis in 220 patients who had anaphylaxis at their index sting and who did undergo immunotherapy and reported the overall incidence of repeat sting anaphylaxis as 56% which was unrelated to the time interval since the initial sting reaction.²⁵¹ Spergel et al examined whether the organ system involved in the initial allergic reaction

predicted the outcome during subsequent food challenge and found that patients typically experienced a similar reaction on re-exposure to the initial reaction.²⁵² In our anaphylaxis group a more severe historical reaction or use of adrenaline was associated with the development of anaphylaxis compared to no anaphylaxis. The fact that only these risk factors have been identified highlight that the severity of a reaction to food is unpredictable and this is likely due to the interaction of patient-specific factors (e.g. degree of sensitisation, target organ reactivity, co-morbid diseases) and event specific factors (e.g. amount ingested, concomitant illnesses). An association between asthma and the likelihood of developing anaphylaxis was not found but has been reported in another study.²⁵³

Late reactions

There is limited information on late reactions following double blind placebo controlled food challenges. Late symptoms occurred in 4% challenges and were mostly generalised cutaneous (86%). Adrenaline was used to treat late symptoms in this study as a precautionary measure but not necessarily because the symptoms were severe or worrying. One person developed respiratory symptoms but this occurred as part of a protracted reaction and was an unusually severe case. In a study of children and adolescents undergoing food challenge by Saleh-Langenberg et al, 20.8% had late reactions however they report a similar frequency of late symptoms on the placebo day as on the active day. The majority of late symptoms reported were those of restlessness, crying and dizziness and cutaneous symptoms were less frequent than our study (33.3%).²⁵⁴ There were no reports in our study of symptoms occurring later than 2.5 hours. The recommended period of observation following DBPCFC varies in clinical practice between 2-24 hours.^{255,256} Given that 96% reactions in our study had resolved by 2 hours, I believe that this is an adequate safety net to ensure that the majority of patients are fit for discharge, however this recommendation would come with the caveat that following episodes of particularly severe anaphylaxis, a longer period of observation may be required.

Perception of severity

The Visual Analogue scores were developed for this study and have never been used previously in clinical practice. They proved a useful tool for recording subjective perceptions of severity. Results showed that there was only a moderate correlation between the investigator's perception of the reaction and the participant's, probably due to the fact that there are differences in the way each party uses the scale. For example the investigator, based on their experience of seeing and treating hundreds of reactions, might believe that only life-

threatening reactions should be awarded a score of 9 whereas a highly anxious patient may regard the development of throat tightness a worrying enough symptom to grade the reaction extremely highly. Thus each party has a different starting point. This explains why looking at within-individual comparison of investigator and participant scores with each visit proved to be a more sensitive comparison. When comparing the investigator's score to the numerical overall severity score there was a high correlation. This is to be expected as the investigator knows the appropriate weight and gravity to apply to certain symptoms. However the investigator VAS, rather than just being a summation of symptoms, can incorporate extra dimensions of the reaction for example response to treatment and nature of recovery. In addition it can provide a more sensitive evaluation of qualitative severity measures particularly in cases where severe and dangerous symptoms were observed, such as the acceleration of symptom progression and interaction between symptoms for example scalp pruritus and sudden onset rhinitis appearing together is a more ominous sign than each symptom appearing separately and transiently during the challenge. Therefore the investigator VAS is a good supplement to the main numerical score. Additional work is needed on the correlation of independent investigators scores and ideally the VAS scores of two investigators judging the reaction contemporaneously should be compared. It was concerning that there was a poor correlation between the participant's VAS and the numerical severity score. This raises the concern that participants are underestimating the severity of symptoms. Pumphrey et al noted that severe reactions are frequently not dissimilar from more mild reactions at onset, so individuals experiencing life-threatening reactions may not initially realize the potential severity.¹⁴¹ This finding is worrying as it may suggest that if patients misjudge their reactions in such a way, it may result in a delay to seeking medical help and administering life-saving treatment.

Two severe anaphylaxis cases

Regarding these participants, there were a few interesting and informative features. In the case of Participant 1, following the administration of the first dose of adrenaline the participant developed a junctional arrhythmia. Cardiovascular features are reported in anaphylaxis including coronary artery spasm and arrhythmias²⁵⁷ as well as the Bezold Jarisch reflex causing a paradoxical bradycardia during extreme hypovolaemia in 10% patients with anaphylaxis.¹⁷⁷ It is difficult to know whether the cardiac arrhythmia occurred as a result of the very severe anaphylaxis he was suffering from or as the result of the administration of intramuscular adrenaline. Cardona *et al* examined the safety of adrenaline use in anaphylaxis

and found that side effects occurred in 21.64% cases and potentially severe adverse effects including ECG alterations occurred only in 2.99% cases.²⁵⁸ In our study adrenaline was very well tolerated with few side effects (data not shown), however, it must be noted that the study participants were young fit adults and the case may be different in an older age group. Participant 2 had significant eczema and it is possible that this played an important role as an additional co-factor in worsening the severity of her reaction. Summers et al found that severe atopic dermatitis correlated with a 3.1 fold increased risk of severe reactions with cardiovascular instability.²⁴⁷ It is possible that the presence of abnormal eczematous skin may have masked early symptoms such as urticaria. In addition, the palmar itching, reported as 'normal' for her at Dose 5, could actually have been a herald sign. Previously Van der Zee et al reported that children with atopic dermatitis react to higher doses of peanut than patients without atopic dermatitis supporting this theory.¹²⁰

Limitations

The major limitation of this study is that reactions were terminated at the onset of a certain number of objective symptoms. Symptoms could therefore not evolve and reactions could not progress to their full potential had intervention not occurred making threshold dose and reaction severity inextricably linked. Therefore it was necessary to restrict analyses to the lowest comparable dose within individuals to circumvent this issue. A better study design is indeed required to answer specific questions about severity and this is described in the chapter, 'Further work'. Another important point is that investigator and participant bias is unavoidable. Once either party has witnessed the first reaction it is possible that their experience of that reaction may influence their actions in the next challenge. From the investigator's point of view this includes lengthening doses intervals knowing how symptoms have previously evolved or earlier delivery of treatment. From the participant's point of view they may anticipate certain symptoms or overplay them. The other possibility is that in the beginning, as the investigator, I acted in a more cautious way and as the study progressed I became more relaxed when managing reactions. By having strict objective criteria for threshold estimation and challenge termination these interactions were hopefully minimised. Lastly, our proposed severity scale is one of the first of its kind for food allergy. Applying a numerical value to a qualitative entity is inevitably subjective. To ensure that the score provides accurate assessment of severity in different populations at different time points validation is required in other patient cohorts.

CHAPTER 6

Is mast cell tryptase measurement useful in food allergic reactions?

Introduction

Serum tryptase is a marker of mast cell activation and a rise in tryptase is a useful indicator of whether an allergic reaction has taken place. Tryptase has been shown to be of value in allergic reactions triggered by venom²⁵⁹ and drugs²⁶⁰ however its utility in food allergic reactions remains unknown. Some studies report that tryptase does not rise in food induced anaphylaxis¹⁶⁷ nor in non-hypotensive reactions. These studies are largely based on data derived from post mortem samples of patients who died from anaphylaxis²⁶¹ or patients presenting acutely to emergency departments resulting in varying sample times and a bias towards more severe reactions. Experimental evidence of tryptase in food-allergic reactions and in particular on its typical time course is lacking.

We present a large prospective study of the diagnostic utility of serum tryptase in experimentally induced peanut allergic reactions of varying severities. Our aim was to establish whether tryptase rises in food allergic reactions, the optimal time point for tryptase sampling and an optimal diagnostic cut off in serum tryptase rises for determining a reaction versus no reaction.

Methods

Participants

Fifty allergic adult participants with a history of systemic reactions (urticaria, angioedema or respiratory/gastrointestinal tract symptoms), with acute onset of symptoms after ingestion (up to 2hours) of peanut were recruited from 2013 to 2016. Allergic status was determined by evidence of sensitisation to peanut demonstrated by positive skin prick tests (SPT) and positive serum specific IgE using ImmunoCAP (ThermoFisher, Uppsala, Sweden) to peanut and peanut components Arah 2 and/or 1 and 3. A positive test was defined using the criteria of ≥ 3 mm for SPT and ≥ 0.35 kUA/L for ImmunoCAP. Ethical approval was obtained from the National Research Ethics Service (NRES) committee East of England. Informed consent was obtained from all participants. We carried out a prospective study of mast cell tryptase

during reaction. All participants underwent an initial peanut challenge. Participants were given incrementally increasing doses of peanut protein until they developed clear objective signs of an allergic reaction. Reaction severity was scored according to the Ewan and Clark severity scale.¹⁵² Anaphylaxis was defined as two or more of the following rapidly occurring symptoms (minutes to hours) following exposure to peanut: generalised involvement of the skin-mucosal tissue, respiratory compromise (pronounced dyspnoea, wheeze, bronchospasm), reduced blood pressure or associated symptoms, severe and persistent abdominal symptoms (cramping abdominal pain and vomiting)²³⁰ and corresponded to Ewan and Clark severity grade 5 reactions. The first peanut challenge was double blind with all patients undertaking a placebo arm. The initial challenge was followed by 3 further open challenges.

Mast cell tryptase- principle and method of the procedure

Blood samples for mast cell tryptase were taken on each challenge day prior to the commencement of peanut doses (pre-challenge sample, at the onset of reaction and at 1 and 2 hours post reaction). Sampling schedule is shown in Appendix 6. On non-reactive challenge days, samples were taken prior to the commencement of doses and at 2 hours post last dose. An initial serum tryptase was also taken for each participant on their screening visit. Samples were taken in a 2ml clotted blood- brown gel EDTA/Heparin plasma tube. Tryptase was measured using UniCAP (Thermo Scientific, Uppsala Sweden) which measures the concentration of free tryptase in the human serum. It is intended for use with the Phadia 250 automated instrument. In this test, the anti-human tryptase is covalently coupled to an ImmunoCap and reacts with tryptase in the patient serum. After washing away non-specific binding molecules, enzyme labelled antibodies against tryptase (conjugate) are added to form a complex. Following incubation, unbound enzyme anti-tryptase is washed away and the bound complex is incubated with a developing reagent. The fluorescence of the eluate is measured when the reaction is stopped. The higher the response value the more tryptase is present in the specimen. To evaluate test results, the responses for the participant samples are converted to concentrations with the use of a calibration curve. The lower detection limit of the assay is 1 ng/ml and higher detection limit 200 ng/ml.

In this study the upper limit of normal was taken to be 11.4 ng/ml (95th centile).

Statistical analysis

Mean and standard deviations were calculated and because of non-normality, median and a non-parametric confidence interval around the median was used. A p value of <0.05 was

classed as statistically significant. Peak percentage rise was calculated as (peak tryptase level during reaction/same day pre challenge sample) x100. Correlations were calculated with linear regression analysis. To investigate the utility of serum tryptase in determining whether an allergic reaction has occurred receiver operating characteristic (ROC) curves were used to establish the cut off providing the best sensitivity and specificity for the test. Statistical analyses were performed with STATA version 12 (StataCorp, College Station, Texas).

Results

Participant characteristics

50 adults aged 18-39 (median age 20.8, M=28, F=22) were included in the study. Tryptase readings were measured in 177 reactive peanut and 45 non-reactive challenges. In 17/177 challenges tryptase levels were <1ng/ml and therefore were unable to be analysed. All participants were reactive and developed objective symptoms during the peanut challenges and non-reactive during the placebo challenges. Fourteen of the 160 reactions (9%) were classed as anaphylaxis. No participants had a raised basal tryptase level.

Tryptase during placebo challenges

Tryptase readings were available for 45 placebo challenges. In the placebo challenges, the percentage change of the 2 hour reading/baseline was zero in 21 cases, positive in 11 and negative in 13. The median peak percentage rise was zero (95% CI 0,0 IQR -1.92, 4.31), the arithmetic mean (which gives higher weight to proportional increases than decreases) was 1.4% and the geometric mean (which gives both equal weight) was -0.1%. Participants with higher placebo 2 hour/baseline rises did not exhibit higher reaction peak tryptase/baseline rises.

Tryptase rise during reactions

Tryptase was raised above the upper limit of the normal range (11.4 ng/ml) in 4/160 reactions and all 4 reactions were severe anaphylaxis. In these reactions the predominant symptom was hypotension in 1/4 and profound dyspnoea with no hypotension in 3/4. Peak levels were 17, 13, 12.2 and 12 ng/ml respectively. In 10/14 anaphylactic reactions the peak tryptase remained in the normal range. The peak percentage rise was calculated for all reactions. Relative to the baseline, a rise was noted in 100 /160 reactions (62.5%); anaphylaxis group 14/14 (100%) non-anaphylaxis group 86/146 (59%). The average peak percentage rise for all reactions was 34.8% (median 25% CI 95% 13.3%-33.3%). In the anaphylaxis group, the

average rise was 148.4% (median 70.8% CI 95% 33.3%-300%) and in non-anaphylaxis group was 23.9% (median 14.0 % CI 95% 0%-25%).

Time course of serum tryptase during reactions

Median percentage change in tryptase over time is shown in Figure 14(a) and (b). Relative to a completely flat time course in placebo challenges, tryptase levels rose over time in positive challenges. Tryptase followed a steeper time course in anaphylaxis reactions versus non anaphylaxis reactions. The commonest time for peak values to occur in both groups was at 2 hours (57% anaphylaxis group and 36.5% non-anaphylaxis group). The same peak readings at both 1 hour and 2 hours were observed in 35.7% of participants in anaphylaxis group and 27.1% of participants in the non-anaphylaxis group. No participant experienced a peak level independently at 0 hours (onset of reaction) (Table 19).

Table 19: Timing of peak tryptase readings (% participants in each group)

	0hr	1hr	2hr	1hr/2hr joint	0hr/1hr joint	0/1hr/2hrjoint	0/2hr joint
Anaphylaxis	0.0	7.1	57.1	35.7	0.0	0.0	0.0
Non-anaphylaxis	0.0	16.5	36.5	27.1	3.5	14.1	2.4

Figure 14(a): Tryptase time course during peanut and placebo challenges. Median percentage rise in tryptase (peak tryptase/baseline) for each time point is shown.

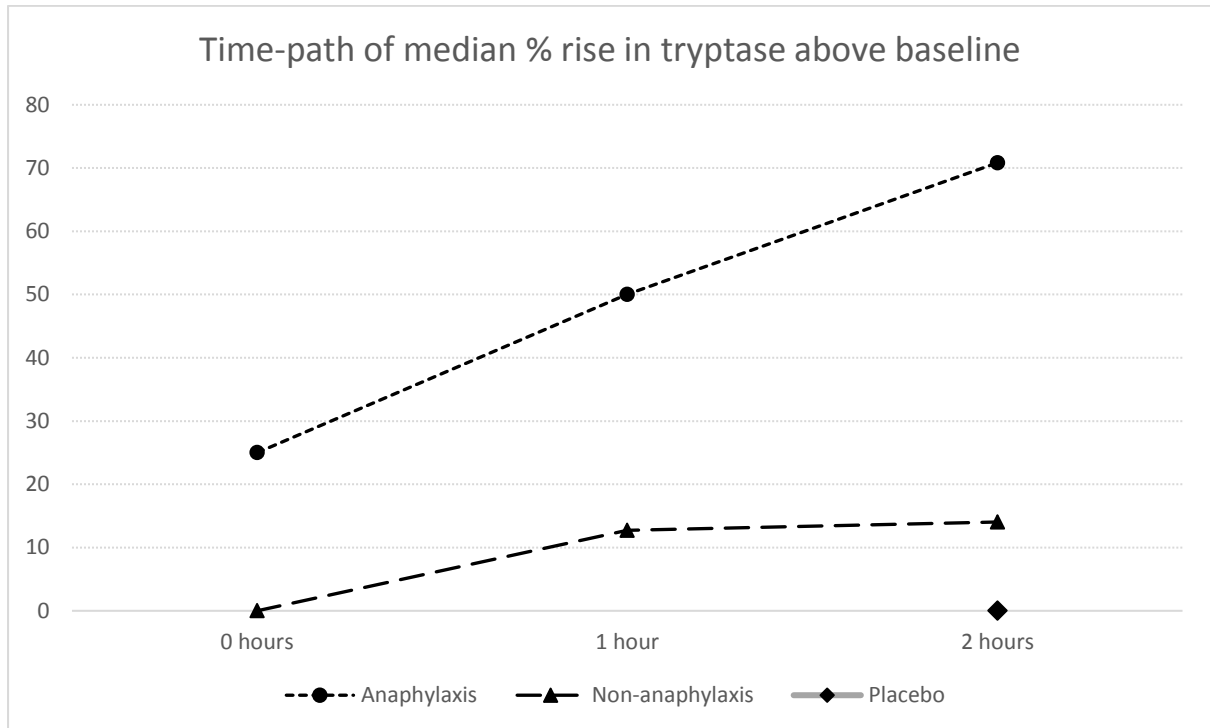
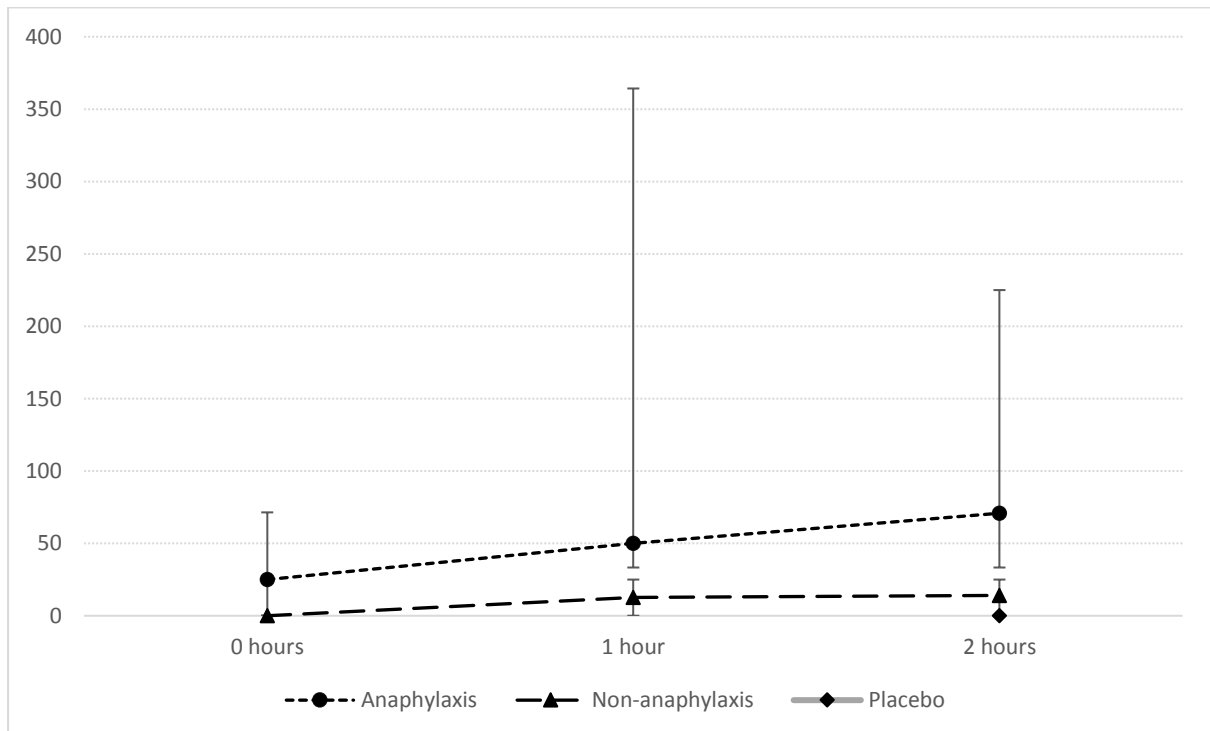


Figure 14(b): With confidence intervals



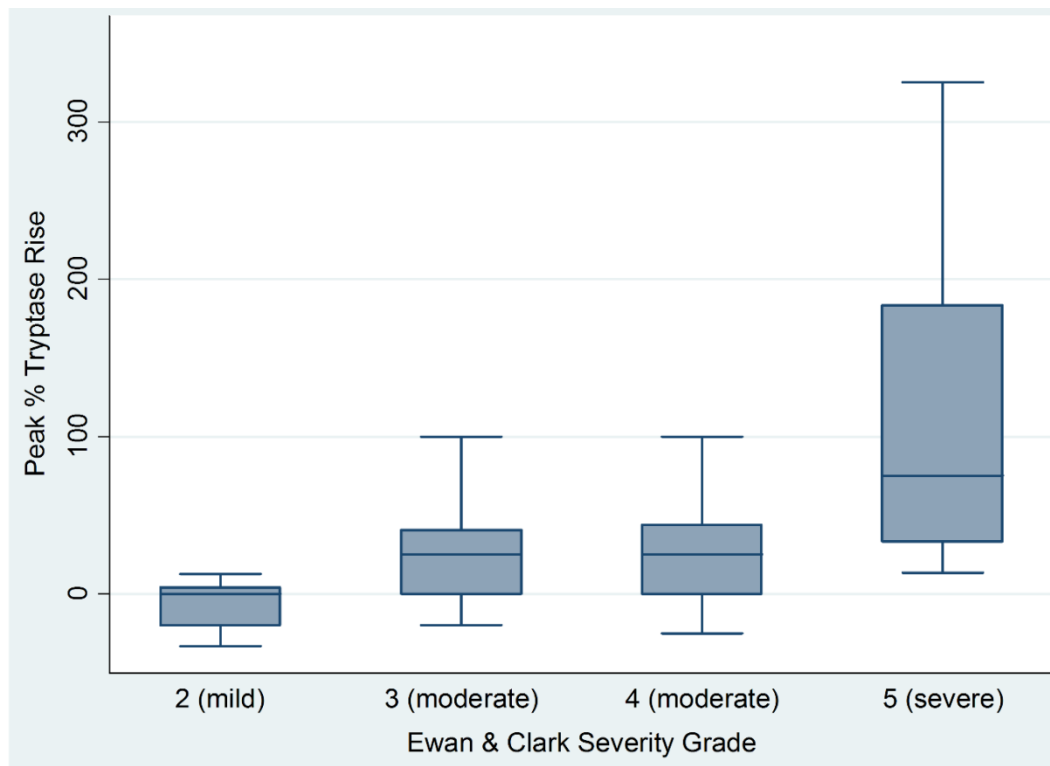
Tryptase in relation to severity

Using the Ewan and Clark severity grading system the reactions were graded as shown in Table 20. Peak tryptase correlated with severity with a correlation coefficient of 0.37 ($p < 0.05$) (Figure 15).

Table 20: Frequency and classification of systemic reactions (n=160) during peanut challenges according to Ewan and Clark Severity Grade.

Classification	Symptoms	Frequency %
1	localised cutaneous erythema/urticaria/angioedema/oral pruritus	0
2	Generalised erythema/urticaria/angioedema	3.1
3	At least grade 1 or 2 and GI symptoms/rhinoconjunctivitis	42.5
4	Mild laryngeal oedema voice change/tightening of throat/mild asthma	46.9
5	Severe pronounced dyspnoea/hypotensive symptoms/collapse/loss of consciousness	7.5

Figure 15: Serum levels of tryptase as a function of the severity of the allergic reaction.



Clinical features associated with a rise in tryptase

Correlations were measured between peak percentage rise in tryptase and binary symptom variables (localised cutaneous erythema, generalised erythema, mild respiratory symptoms, moderate to severe respiratory symptoms, abdominal pain/nausea, vomiting, throat tightness, hypotension and dizziness/tachycardia with no hypotension) based on within-participant variation. Statistically significant positive correlations were found with moderate to severe respiratory symptoms which were associated with an 84.5% higher peak percentage rise in tryptase (CI 95% 1-168% $p < 0.05$) and dizziness associated with 16.7% higher peak percentage rise in tryptase (CI 95% 0.4-33% $p < 0.05$).

Within participant variation in baseline tryptase over time

Inter-day within-participant variation of baseline was summarised by averaging measures of within-participant variation across all participants. Two hundred and fifty nine baseline samples were included in the analysis: 50 screening, 160 on the day pre-challenge and 49 placebo samples. The average participant mean baseline was 3.74ng/ml, based on an average of 5 observations. The average participant standard deviation of baseline was 0.46ng/ml, giving an average coefficient of variation of baseline of 0.14 corresponding to baseline variation of 14%. The average interquartile range of baseline was 0.59 and the average max-min range was 1.08.

Relationship of baseline tryptase levels to reaction severity

No correlation was found between a participant's median baseline tryptase level and the grade of their most severe reaction (correlation coefficient -0.09 $p = 0.54$). No baseline tryptase was raised > 11.4 ng/ml.

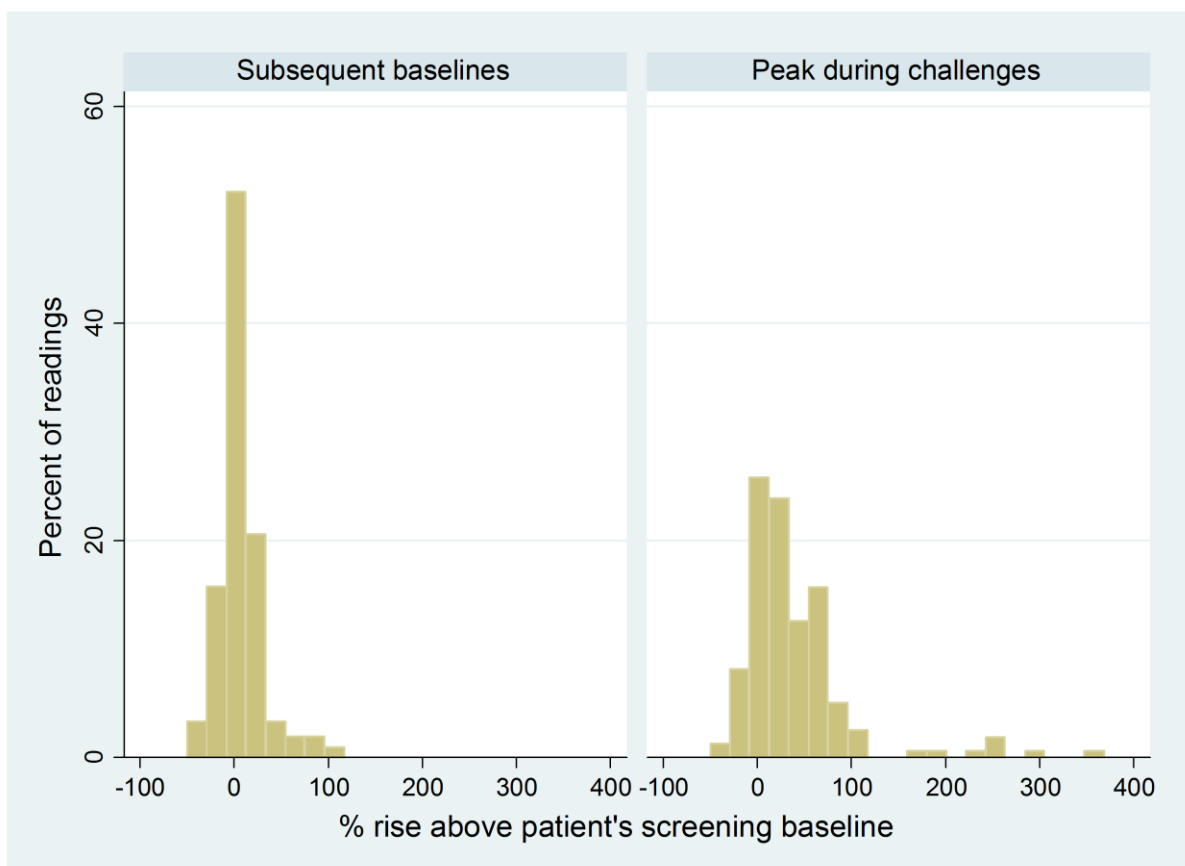
Clinical utility of serum tryptase as a diagnostic marker

A ROC analysis was performed to identify the optimal cut off point for percentage rise in tryptase above baseline to distinguish between a reaction and no reaction in our population, taking into account inter-day within-participant baseline variation. A clinician measuring tryptase acutely during a suspected reaction may compare this reading to a baseline taken at clinic either on a previous occasion or during subsequent assessment. The diagnostic

usefulness of the acute reading depends on whether the difference between reaction and non-reaction (baseline) readings is large relative to inter-day variation in baseline readings.

Thus the two following distributions were compared in the ROC analysis: i) peak tryptase rise (160 reaction observations) relative to screening baseline and ii) subsequent day baselines relative to screening baseline (209 observations) the distributions of which are shown in Figure 16. The 209 subsequent baseline observations are clustered around zero and symmetric while the reaction observations exhibit far fewer zeros and are markedly skewed positive.

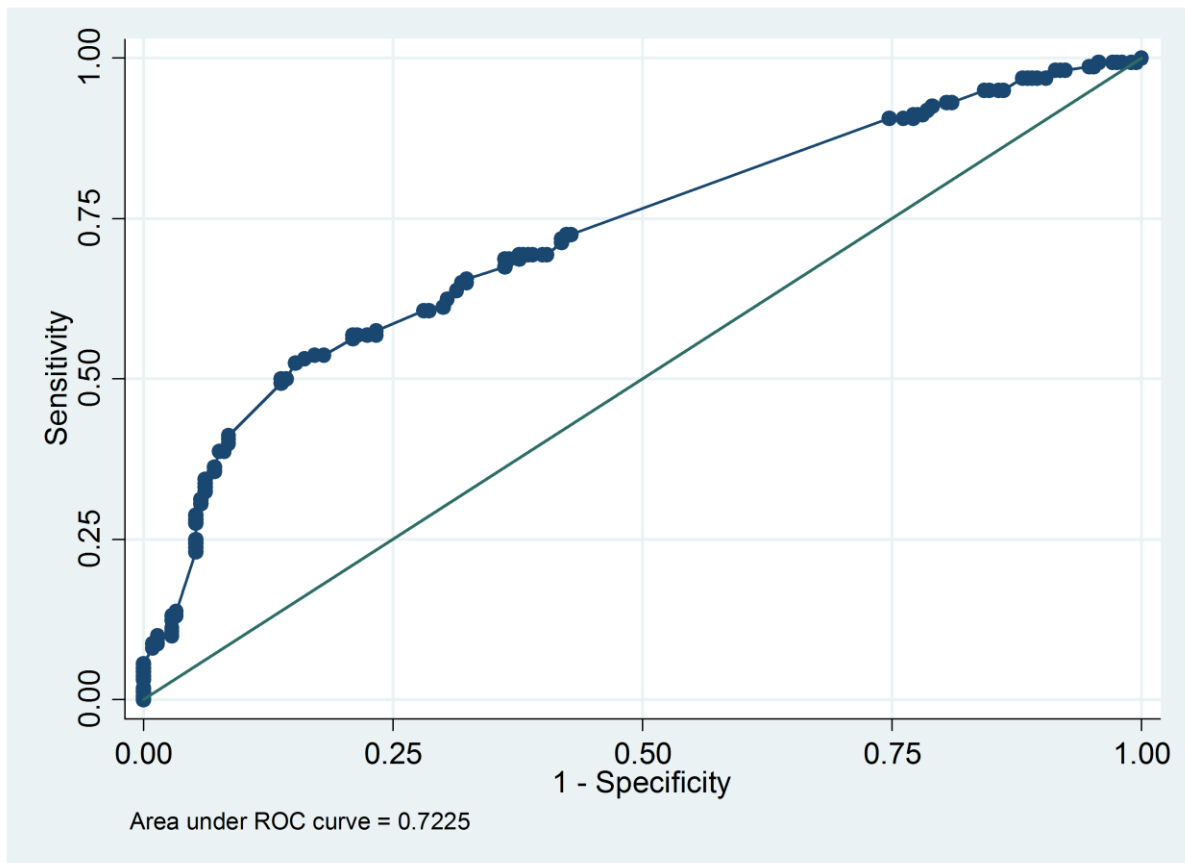
Figure 16: Histogram demonstrating % change relative to initial baseline in reaction versus no reaction.



Combining these 369 observations, the area under the ROC curve was 0.72 (95 CI 0.67-0.78), indicating that percentage tryptase difference relative to a different-day baseline is potentially useful as a test to identify the presence of a reaction in a clinical context. The Youden Index, which weights the cost of false positives and false negatives equally, identified the optimal

cut off to identify a reaction as a 30% rise, which was associated with a sensitivity of 0.53 and specificity of 0.85. (Figure 17). As an alternative approach to identifying an appropriate cut off, taking the two standard deviation within-participant % variation in baseline above the participant specific mean baseline (which represents a 95% upper confidence bound on percentage baseline variation) yields a cut off of 28%, which is close to the 30% derived from the ROC analysis. Taking the median baseline value of tryptase for the median subject (4 ng/ml), a 30% rise equates to a 1.2ng/ml increase in serum tryptase to 5.2ng/ml.

Figure 17: Receive operator characteristics (ROC) curve analysis to obtain the best value of ratio between peak tryptase during reaction and initial baseline serum tryptase to distinguish a reaction from a non-reaction.



Discussion

We have conducted the largest prospective study on serum mast cell tryptase during experimentally induced peanut allergic reactions. We provide new data on tryptase time course during peanut allergic reactions and establish a diagnostic cut off to determine whether a food allergic reaction has taken place.

Previous studies have disputed the sensitivity of serum tryptase during a food allergic reaction stating that tryptase often fails to rise acutely. Indeed, using only the established upper bound (95th centile) of the tryptase assay to denote a rise in tryptase (>11.4ng/ml) this statement is correct. Tryptase only rose above this level in 2.5% allergic reactions in our study. Previous studies on venom allergy have demonstrated that the sensitivity of the test can be improved if acute levels are compared to a baseline tryptase level taken after the reaction has resolved.^{188,262} This method detects rises which may occur within the normal range of the assay. When adopting this approach we observed a rise in tryptase compared to the baseline in 62.5% all reactions and in 100% of anaphylactic reactions. Reactions in which tryptase failed to rise were distributed across all severity grades and were not necessarily the least severe reactions.

The amplitude of the tryptase rise is known to correspond with the allergic trigger. High peak tryptase levels are observed in allergic reactions involving anaesthetics, intravenous drugs and venom with smaller peaks observed in food allergic reactions.²⁶³ This likely due to the former being associated with large doses of allergen being rapidly delivered directly into the systemic circulation with resultant extensive cutaneous and perivascular mast cell degranulation and mediator release. Hypotension is often a presenting feature of these reactions. This contrasts with food allergic reactions where allergen absorption occurs at a slower rate across the oropharyngeal and gastric mucosa and hypotension is rarely a feature⁸⁴ thus the serum rises are likely to be much smaller. Minimal rises in tryptase may also reflect the limited extent of mast cell activation for example the gastrointestinal tract versus entire skin. Furthermore upon release, mast cell tryptase may be secreted into locally involved organs rather than the systemic circulation for example into the gut lumen in predominantly gastrointestinal reactions or into laryngeal / bronchial secretions in respiratory reactions. Other possible explanations for this could be differences in mediator content of skin versus mucosal mast cells. Schwartz et al reported that mucosal mast cells contained less tryptase

compared to skin mast cells.²⁶⁴ Lastly it may be that changes in tryptase were too small to be detected by the assay, particularly if the participant had low starting levels of serum tryptase for example <1ng/ml. It has previously been postulated that the failure to rise include a predominant basophil rather than mast cell involvement in an acute allergic reaction.¹⁶⁷ Basophils are known to contain less than one hundredth of the amount of serum tryptase contained by mast cells.¹⁷⁸

Other studies are often based on tryptase levels of participants presenting to emergency departments or post mortem samples and are therefore biased towards more severe reactions. Our study represents a wider spectrum of reactions including data on reactions of mild to moderate severity, 92.5% reactions in our sample. We have shown, in line with previous studies, that tryptase rise correlates with an increasing reaction severity.²⁶⁵ It has been shown previously that severe anaphylaxis is more likely to be associated with higher tryptase levels¹⁹² and peanut anaphylaxis is no exception. In our study the mean rise in tryptase in the anaphylaxis group was 148.4% above the baseline level in contrast to 23.9% in the non-anaphylaxis group. Previous studies have attempted to relate individual symptoms to mediator release. Lin et al showed an association between the presence of tachycardia and urticaria and a rise in tryptase.¹⁸⁶ Similarly in our study we showed a significant relationship between the presence of dizziness and tachycardia and tryptase rise. Both of these are likely precursors to hypotension. We are the first to demonstrate that the presence of moderate-severe respiratory symptoms is associated with a higher tryptase. It follows that tryptase release most likely occurs mainly in the airways in respiratory predominant reactions. Regarding baseline tryptase as a predictor of reaction severity, Sahiner et al showed that serum baseline tryptase levels may predict moderate to severe anaphylaxis in children with food allergy.²⁶⁶ We have not been able to prove a correlation between baseline tryptase levels and reaction severity. Our study, however, did not include any participants with raised baseline levels of tryptase and other studies in venom allergic adults have established a clear relationship between elevated baseline levels of tryptase and severe anaphylaxis.²⁶⁷

Knowledge of tryptase kinetics is crucial in determining optimal sample timing to capture peak levels. Unlike other mediators such as histamine which are detectable within minutes of the onset of allergic symptoms, tryptase may not be detectable during the first 15-30 minutes.¹⁸¹ In anaphylactic shock the increase in tryptase level in blood occurs later than the onset of shock and rash and well after the histamine peak.¹⁸² Therefore the appearance of tryptase in the blood likely reflects mast cell activation in a variety of tissue locations but is

unlikely to be the cause of anaphylactic shock.¹⁸³ It has been demonstrated that tryptase potentiates histamine release.²⁶⁸ Mature tryptase is released in a complex heparin scaffold thus slowing its release.¹⁸⁴ Once in the circulation, the half-life is about 2 hours and levels are thought to diminish after this time. It is recommended therefore that the optimal time for sampling is between 30 minutes and 2 hours after the onset of a reaction. This recommendation was largely based on a hypothetical time course proposed by Schwartz following their observations on 3 patients undergoing bee sting challenges and also 39 patients who developed anaphylaxis following a yellow jacket or honey bee sting.²⁶⁹ In this latter study by Van der Linden, however, tryptase measurements were only taken up to 60 minutes with no further readings.²⁵⁹ Previously we have studied tryptase time course in anaphylaxis due to general anaesthesia and found that peak tryptase levels in paired samples taken at 0 to 1 hour and 1 to 2 hours were statistically similar.²⁷⁰ In the current study we also observed that peak levels did not occur immediately after the onset of reaction. However in contrast with other studies where peaks have been observed in the majority of patients within 30-60 minutes of reaction onset,¹⁸⁵ we noted that peak levels occurred later at 2 hours in the anaphylaxis group. One participant with severe anaphylaxis had a further reading taken in his recovery period at 3 hours which showed even higher levels compared to 2 hours. Unfortunately no data on tryptase readings from 2 hours onwards was available in the remaining participants. Furthermore the degradation pathways of tryptase particularly in the extracellular environment is not known, it is possible that there is variability in the clearance of tryptase between individuals.²⁷¹

Limited data exists on the intraindividual variability of baseline tryptase over time. Generally it is believed that serum tryptase remains stable. We showed that there was a variation of 14% in baseline tryptase readings over time within an individual. Some studies also suggest that there may be a diurnal variation in tryptase of up to 15%²⁷² however we saw little variation in tryptase measurements taken over the course of the day during placebo challenges.

Having accounted for this inter-day variability we sought to establish a cut off in tryptase rise above baseline to signify an allergic reaction. Currently there is no consensus on the optimal cut off for diagnosing either mast cell activation or anaphylaxis with different studies quoting different values. A cut off point of 11.4ng/ml is recommended by the commercial company Thermofisher for the Immuncap assay based on this value being the 95th centile of tryptase levels in 129 non allergic control subjects.²⁷³ Valent *et al* state that the acute serum total

tryptase level should be an increase 20% plus 2ng/ml over the baseline level of tryptase to be indicative of mast cell activation.²⁷⁴ Enrique et al used absolute tryptase levels rather than a percentage rise to establish a rise of 8.23ng/ml as a cut off to identify anaphylaxis in patients.²⁷⁵ Other studies have defined a threshold difference of 2.0ng/ml or more based on sting challenges during venom immunotherapy.¹⁸⁵ Egner *et al* studied tryptase during general anaesthesia reactions and established a threshold increment of 20% in tryptase to identify mast cell mediator release in an additional 14% of cases with peak tryptase between 5 and 14 ng/ml and a further 15% with levels below 5ng/ml.²⁷⁶ Data on cut offs specifically for food allergy are scarce. One study by Wongkaewpothong identified a delta-tryptase cut-off of >0.8 µg/L to confirm anaphylaxis in shrimp induced reactions.¹⁸⁹ In our study, we identify an optimal cut off of a 30% rise in tryptase above baseline to signify an allergic reaction in peanut challenges.

The shortcomings of this test also need to be noted. The result of a serum tryptase sample is not immediately available at the time of a reaction and usually takes a few hours to return. Thus although this test is extremely useful post reaction to confirm that mast cell activation has occurred, contemporaneous diagnosis is still reliant on the attending clinician to identify the signs and symptoms of an allergic reaction. Further research is required to develop tests for biomarkers which can provide results immediately at the time of reaction.

Our study has some limitations. Patients were discharged when they had fully recovered and this was almost always within 2 hours of the onset of reaction. Therefore we lack tryptase data on time points greater than 2 hours post reaction. This is needed for a more complete time course and to corroborate exactly where peak levels are occurring. However data on general anaesthetic reactions show a substantial fall in tryptase from 2 to 4 hours, with the exception of those with extremely elevated peak tryptase levels of greater than 200 ng/ml.²⁷⁰ Further it would have been useful to measure other allergic mediators such as histamine or PAF in order to validate our tryptase findings with regard to mast cell activation. In order to further improve diagnostic sensitivity we could consider measuring only β tryptase, as this is only released during degranulation and is therefore pathognomonic of this phase. However this is not currently available for diagnostic use and validation of sensitivity and specificity is needed before it is used routinely. In conclusion acute tryptase measurement is of value in food allergic and normotensive reactions. There is a rise during reactions but this is mostly within the normal range. It was raised above the normal range on only 4/160 reactions thus a single acute measurement of serum tryptase has poor sensitivity in the diagnosis of peanut

allergic reactions. In food allergy, tryptase peaks are relatively low and thus relating reaction levels to baseline levels is essential to capture a rise. We show that tryptase peaks may occur later at 2 hours in food allergic reactions, therefore readings both at initial and the 2 hour time points are important. We propose a cut off in tryptase rise which may guide clinicians in establishing whether mast cell activation and thus an IgE mediated food allergic reaction has occurred.

CHAPTER 7

Conclusions and Future work

Ongoing work

Immunological studies

Allergen immunotherapy to peanut induces immunological changes including suppression of effector cells and alterations in the amount of circulating allergen specific antibody.²⁷⁷ Over time, during the immunotherapy course, there is an initial rise then a gradual fall in peanut specific IgE. Furthermore, with repeated daily oral administration of allergen there is an increased production of specific IgG, IgG4 and an inhibitory IgG-dependent serum factor which inhibits the allergen/IgE binding response.²⁷⁸ Santos et al noted that patients who had a higher ratio of peanut-specific IgG4 to IgE reacted to higher doses of peanut protein during challenge suggesting that IgG4 competed with IgE for binding to allergen and blocking its effect.¹²³

Given the reduction in threshold and rise in severity with successive challenges observed in this study, supportive work is being undertaken to examine whether immunological changes take place over the course of four oral challenges. The hypothesis is that that specific IgE levels rise over time and IgG4 levels may fall. Arah2 specific IgE and IgG4 levels are being measured in plasma samples which were taken at each of the four repeat challenges for 30 participants.

Future work

Studying cumulative versus discrete doses

Food challenges remain the gold –standard outcome for interventional clinical trials in food allergy, of e.g. oral immunotherapy. However, uncertainty exists whether cumulative and discrete dose thresholds are comparable. Providing clarity on this issue will aid companies developing interventional drugs for food allergy where oral challenge is used as a primary outcome. Drug regulators in turn required confidence that cumulative dose thresholds obtained in drug trials represent discrete exposures in real-life. Eliciting doses are expressed differently for different studies. In some studies discrete doses are quoted which is the lowest individual dose causing a reaction.¹¹⁹

Other studies, like ours, cite the cumulative dose up to the point where the individual has reacted.²⁰⁶ The former approach assumes that the dose is digested and absorbed in a given interval and treats each dose as an independent event with each dose having little influence subsequent doses whereas the latter approach simply assumes that all exposures in a specific time frame are summed. There are currently no data to suggest one approach over another, however using a discrete dose over a cumulative one will lead to a more conservative and possibly more restrictive approach when attempting to model population thresholds.

Therefore given that in this study we have data on cumulative doses it would be useful to examine whether giving patients a single discrete dose would induce similar symptoms to those which occurred following incremental up dosing to a total dose.

Examining severity across participants

An approach to managing the endogeneity between dose and severity, would be to give all participants the same single dose and measure the effect of that dose on symptoms. For example participants who had reacted at a cumulative dose of 133.333mg would be invited back and given this quantity as a single dose.

Sampson has postulated that the longer it takes for anaphylactic symptoms to develop the less severe will be the overall reaction.¹⁵³ This could be formally tested and the time of onset of each symptom would be recorded and the time treatment administered will be recorded. This will give clearer information on how severity varies across individuals and may indicate differences in absorption patterns across individuals.

It is possible that differences in timing of onset of symptoms and differences in symptom pattern occurs as a result of genetically determined differences in mast cell activation, mediator release profiles or differences in tissue responses to such mediators. Furthermore the distribution of sensitised effector cells will influence organ involvement in anaphylaxis and symptom progression.

Examining the reproducibility of threshold over time

Given that the first challenge of the study was double-blind placebo controlled and the repeat baseline (no intervention) challenge was active arm only, comparison of thresholds between the two is problematic as 'like for like' challenges are not being compared. In order to answer the question as to whether there is consistency in the challenge threshold dose with a repeat challenge, a further no-intervention challenge could be repeated under the same conditions as

the first. This more accurately determine background variation in threshold over time without the application of co-factors. Ideally participants should be challenged at the same time of year as their original challenge so that confounding factors such as seasonal rhinitis are controlled for.

Examining physiological changes during sleep and exercise

Original study participants can be further studied to examine whether gut permeability changes during sleep deprivation or exercise. Markers of intestinal damage and intestinal permeability including I-FABP and urinary Lactulose/rhamnose could be assessed whilst the participant undergoes challenge with the placebo matrix.

Comparison of symptoms: adult versus paediatric population

Summers et al observed in their study that adults were more likely to experience severe pharyngeal oedema, bronchospasm and reduced consciousness than children.²⁴⁷

I will examine data from a study in London where children were given peanut and tree nut challenges using the same dosing escalation and similar stopping criteria to our study. I will compare symptom frequency, patterns and progression in the two groups examining for differences between the two groups.

Study of further co-factors

Non-steroidal anti-inflammatory drugs such as aspirin have previously been described as cofactors. Proposed mechanisms include tight junction damage, decreased PGE1 and augmentation of mast cell degranulation via Syk kinase.¹³² Peanut challenges could be repeated after participants are pre-medicated with a dose of non-steroidal such as aspirin. Alternatively the effect of alcohol could be studied and peanut challenges could be undertaken following ingestion of a specific volume of alcohol.

Perception of severity across investigators

Van Erp *et al* reported that there is variability in the interpretation of clinical symptoms by clinical experts when they asked to retrospectively assess score sheets from challenges.¹¹¹ Using the Visual Analogue Scale proposed in our study we could determine prospectively whether there is a good intra-observer agreement when scoring food challenges. This would require two observers simultaneously and independently scoring a food challenge.

Further definition of eliciting dose

The majority of participants at baseline challenge reacted at doses 5 and 6 (cumulatively 33.333mg and 133.333mg). This is a relatively large increment from one dose to the next. Given that it is likely that the majority of peanut allergic individuals' thresholds lie in this interval, additional increments could be added in this interval (for example 63.333mg and 93.333mg) for a more definitive determination of the population threshold.

Severity scoring

The numerical severity scale proposed in our study requires validation in another larger participant cohort. Current scores offer poor discrimination between differing severity grades and are not granular enough to capture shifts in severity. A further extension to the severity work in this thesis is to demonstrate the unsuitability of existing scores to be used in this regard. Another working group (iFAAM) has attempted to model a numerical score developed with mathematical modelling (nFASS) which has not yet been published. It would be useful to compare this score with our own.

Conclusion

This thesis provides novel data on several important aspects of peanut allergy which will influence the care of allergic individuals.

A clear threshold of peanut has been identified which induces an objective reaction in a defined proportion of the peanut allergic population. In other words, a level has been identified which is protective for the majority of peanut allergic individuals. Moreover the ability of everyday factors such as exercise and sleep deprivation to influence allergic reactions both with regards to threshold and severity has been clearly demonstrated. This study is the first to establish population eliciting doses for peanut when participants are deliberately subjected to the co-factors sleep deprivation and exercise. Further, we are able to relate these to a reference threshold when no co-factor (no-intervention) is applied to calculate the magnitude of the effect. Thus, compared to a mean ED₅ of 11.8mg peanut protein when no co-factor is applied we show that exercise lowers the ED₅ to 3.8mg and sleep to 1.9mg equating to factors of 0.32 and 0.16 respectively. Currently for allergen risk assessment, an eliciting dose is chosen (e.g. ED₁ or ED₅) which is an exposure that is likely to be without appreciable risks of deleterious effects for a population. Single dose peanut challenges have recently been performed to validate the proposed ED₅ for peanut of 1.5mg peanut protein. Further studies of this kind are required to validate proposed ED₅ and ED₁ doses, particularly in the adult population. Industry can then use these validated reference doses to develop guidelines for the use of voluntary precautionary food labelling. Previously a reference dose of 0.2mg peanut protein, based on the ED₁, has been proposed by the VITAL group. However, the group acknowledge in their study that further application of an uncertainty or safety factor to this reference dose may be necessary to account for individual factors which may potentially affect this dose estimate. Because of a paucity of clinical data, the application of safety factors has followed toxicology practice account for (10-fold) inter-species (for thresholds defined in non-human models) and (10-fold) intra-individual variation in response. In practice, such large safety factors result in very low reference doses which, being near or below the limit of detection of available assays, are difficult to measure with accuracy, rendering them impractical for the food industry to implement. This study, commissioned by the UK Food Standards Agency (FSA), through determining the magnitude of variability caused by co-factors (accounting for intra-individual variability without co-factors), showed that the safety factor can be many magnitudes smaller. Providing thresholds which are more feasible to measure should encourage better industry engagement with

evidence-based voluntary food labelling reducing excessive, overly cautious precautionary allergen labelling.

By experimentally inducing repeated allergic reactions within and across individuals, I have been able to closely observe reaction patterns and severity. I have been able to prove that there is an increase in severity with repeated challenges at regular intervals. This will have clinical implications when advising patients about frequent accidental exposures.

Furthermore by demonstrating that there is homogeneity in symptom pattern from one reaction to the next, patients may be reassured that subsequent reactions are likely to follow the same pattern and progression. It is worrying that a discordance was observed between a participant's perception of their allergic reaction and the overall challenge severity score perhaps suggesting that patients misread their symptoms. This emphasises the need for clinicians to ensure that patients are well informed about features of severe reactions and the indications for adrenaline use.

Lastly the usefulness of mast cell tryptase has been disputed in food allergy. Previously it has been reported that tryptase does not rise in food-induced anaphylaxis and is therefore not useful in assisting diagnosis in acute allergic reactions due to food. Through the first systematic study of its type, this thesis demonstrates that tryptase does rise, albeit within the normal range, necessitating the need for acute levels to be compared to baseline levels. In this study a cut off rise has been determined, identifying an acute allergic reaction whilst taking into account normal variation in basal tryptase. This has significant implications in helping to diagnose whether an acute food allergic reaction has occurred, particularly for physicians treating patients in an emergency setting or for example, during diagnostic food challenges.

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